# White grubs (Coleoptera: Scarabaeidae) associated with Nepalese agriculture and their control with the indigenous entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin

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## ABSTRACT

White grubs became increasingly difficult pests in Nepal for the last few years. Their infestation has been reported throughout the country and magnitude of the problem has been widespread over the past years. In majority of the farming situation, control of these pests are largely abandoning because of the lack of control over their damages. In general, the management strategy depends primarily on the use of highly poisonous poor graded chemical pesticides. The use of bio-control agents in general and fungal based mycoinsecticides in particular are lacking in the country. The former practices have further aggravated the pest problem resulting in wider reluctances for the cultivation of major cereals as well as cash crops in upland farming. With a view to address these issues, an alternative control measures based on fungal antagonist with the fungus *Metarhizium anisopliae* in Nepal was sought since 2003 with the initiation and financial support of Helvetas/ Intercooperation through the coordination of Sustainable Soil Management Programme (SSMP-Nepal).

The research was prompted by the serendipitous findings of *M. anisopliae* and *Beauveria bassiana* in Nepal from infected white grubs and soils during an exploratory study at Institute of Agriculture and Animal Sciences (IAAS), Rampur. Insect pathogenic fungi, *M. anisopliae* (green muscardine fungus) and *B. bassiana* (white muscardine fungus) were identified by a Swiss scientist, Dr Siegfried Keller for the first time in Nepal. With the hope of gearing up of the further works, research plans were drafted, collaborating organizations were identified, and financial supports were solicited and permitted. The research was intensively supported and monitored at various capacities by Dr Siegfried Keller, Senior Scientist, Agroscope FAL Reckenholz, Zurich, Prof. Dr Peter Nagel, University of Basel, Dr Dirk Ahrens, Germany and Dr Philip Kessler, Switzerland through their visits and electronic contacts. This thesis examines the effect of the indigenous insect pathogenic fungus *M. anisopliae* against white grubs in Nepal through a series of laboratory and field experiments. Each of the stages in the development and optimization of a mass production technique for the field assessment is discussed.

Two kinds of initiatives such as exploratory initiatives and exploitation initiatives were undertaken during the study. Both groups of initiatives are interlinked and mutually contributing to each other. The former initiative includes the search of the indigenous insect pathogenic fungi from the soils and insects mainly from white grub prone areas of Nepal. The activities placed under the exploitation initiatives include development of the technology of production and application of insect pathogenic fungi as a means of white grubs control in particular and pest insects in general. In order to conduct the laboratory work, the Insect Pathology Laboratory with a modest level of facilities has been established within the premises of the Entomology Department of IAAS, Rampur of Tribhuvan University (TU). Several dozens of isolates of the insect pathogenic fungi *M. anisopliae* and more than half a dozen of *B. bassiana* were recovered from natural soil and diseased insects using a selective medium and the *Galleria* bait method (GBM). They have been maintained at IAAS, Rampur and as a security copy at FAL Agroscope Reckenholz, Zurich. Isolation, maintenance, mass production and efficacy tests with virulent strains of *M. anisopliae* were conducted. Identification of the damaging species of white grubs was one of the pre requisite for the development of effective control measures. Dynamics of the common beetle species were carried out through light traps and field sampling. They were

later identified based on morphological grounds. A three-tired screening strategy: exploration of insect pathogenic fungi in the farmers' field, pathogenicity assessment under laboratory conditions and its exploitation in farmer's field in Chitwan, Nawalparasi, Tanahun and Parbat Districts of Nepal has been initiated.

Exploratory study revealed that *M. anisopliae* is widely distributed in the Nepalese soils and in insects, however, with low density of *B. bassiana*. Selective medium has proved to be suitable medium for fungus growth and maintenance. Host passages are needed for retaining the virulence. Disease prevalence of *M. anisopliae* in grub cadaver was between 0 and 2% depending on host origin and species and *B. bassiana* was found only from a few soil samples. Analysis of soils from different regions showed that *M. anisopliae* is common and was present in about 50% of the samples irrespective of their origin.

Screening of fungus isolates in time mortality studies indicated that eight isolates gave over 80% infected grubs, sixty five isolates gave over 50-60% infected grubs and rest of the isolates resulted in a low mortality. Five isolates were identified as highly pathogenic ($p<0.001$) against third instar larvae of *Maladera affinis* Blanchard in a concentration of $10^7$ spores /ml. Based on infection rates, the fungus isolates M1, M6, M18, M48, and M50 were found aggressive as compared to rest of the strains. The LT50 of all isolates varied between 2-9 weeks, 12 isolates were highly virulent with an LT50 of 2-4 weeks, 34 isolates had a moderate virulence with an LT 50 of 5-6 weeks and 22 isolates had a low virulence. It is interesting to note that, isolates M1 and M6 were found comparatively more virulent because they killed the larvae reasonably shorter period of time as compared to others strains. These five virulent isolates were further studied in dose mortality assays using conidiospores and blastospores in respect to mass production.

The onset of cumulative mortality and mycosis with different dosages suggested that grubs were moderately to highly susceptible to the fungi with all the dosages, however, higher dosages ($p< 0.001$) were more effective as compared to lower dosages as could be expected. Pathogenicity of conidiospores and blastospores against three different instars of white grubs showed that second instar larvae were more ($p<0.001$) affected than first and third instars. The information regarding the pathogenicity to different stages of the insect would be helpful in targeting their vulnerable stages. Based on time-mortality and dose-mortality studies, M1 strain of fungus was selected for mass production for field application.

Comparative studies of the fungus production in different types of polybags such as polybags with Swiss origin and Nepali origin as well as studies with solid substrates using peeled kernels of barley, rice and wheat were conducted. In all cases, a total of five virulent strains of *M. anisopliae* were tested initially and M1 strain was found superior amongst them. Assessment between bag quality convincingly showed the marked difference between the Swiss polybags and Nepali polybags ($p<0.001$) with a better quality of the fungus in the former types of bags. Similarly, barley kernels are found to be a better substrate for fungus production ($p <0.001$) than rice and wheat. The fungus colonized grains originating from Nepali bags were heavily contaminated irrespective of the solid substrates. In the same way fungus propagules produced as blastospores were better than conidiospores in terms of purity and grain colonisation. The study has indicated the

opportunity of producing barley grains fully colonized with the fungus as a means of controlling white grubs in Nepal.

The results of the field experiments revealed that the fungus can infect the grubs, however, the infection rate were found very low (15-20%) and this parameter remain insignificant when tested at different dosages. In contrast to the infection rate, the establishment of the fungus after application into the soils was found highly significant ($p<0.005$) since the colony forming units (CFU) differed greatly while comparing their density before and after application. In general, both the infection rate and fungus density remain shortly until seven weeks and did not lasts at the same extent until crop harvests (thirteen weeks). This result clearly demonstrated that several factors are responsible for the spread and survival of the fungus in the soil. The density of the grub and soil temperature might have attributed the result since the grub density was reduced three months after sampling. In addition to these factors, the virulence of the BCAs, application and assessment methods, and application seasons may play a great role. Based on this information, *M. anisopliae* can be applied as an important component for white grub management with some modifications. We have to improve our method of production of the fungus, its handling, timing of application and frequencies etc. The persistence and survival of the fungus in the soils are other aspects which need to be considered. Higher mortalities could be achieved using either a white grub's attractant with any preferable crop root feeding or feeding with grains to lure the white grubs to areas of high spore concentrations, or to position *M. anisopliae* granules or spores in the soil so that white grubs would pass through them in their normal patterns of movement such as from lower soil layers to upper layers in the spring and vice-versa in summer. The luring materials would have positive effect in attracting the grubs.

Monitoring studies of white grubs clearly indicated that various species of white grubs are involved in crop damages. Normally, the beetles in almost all the studied areas were active during May to July coinciding with (near) crop sowing, early vegetative growth and harvesting stage of maize. In low belt (terai region) of Nepal, the flight of large number of beetle species occurs in two peaks probably because of the short and overlapping generations of the larvae compared to mid hill regions. In mid hill areas, few species of beetles occur regularly in the same crop field, whereas few other beetle species were common at alternate year probably because of the longer life cycle of the larvae. This pattern suggests there might be involvement of univoltine and multivoltine species in the same locality. There are two peaks of occurrence of the annual beetles the first being March-April and second in June-July. In 2004 in Gunganagar research site, the highest catches of the beetle were found in May (5094) followed by April (2324), whereas, very few beetles were caught in Gaindakot research site. At this site, the highest number was 216 and 118 in May and April respectively. Based on their occurrence, *Maladera affinis* Blanchard being the most frequent (21.2%) followed by *Allisonotum simile* Arrow (19.2%) in Gaindakot research site. In the same way *Adoretus lasiopygus* Burmeister was the most frequent species (51.4%) in Gunganagar followed by *Anomala dimidiata* Hope (6.3%). Similar trends in the occurrence of the beetle were observed in 2005, however, with very low number of catches. Light traps were found one of the effective tools for monitoring phototropic beetles and the information generated from these studies are useful in planning the beetle management programme. Therefore, application of the fungus in any locality should be carried out based on sampling of the white grubs.

In order to understand the natural mortality factors associated in regulating insect population, life table studies and life cycle studies were conducted in 2003/04. These study sites represented low hill area, low mid hill area and mid hill area of Chitwan, Tanahun and Parbat Districts of Nepal respectively. The major objectives of these studies were to know the disturbing factors of white grubs and duration of insect instars and stages so as to plan the microbial control programs in such areas. The life table revealed some natural antagonists such as fungi, *M. anisopliae* and *B. bassiana*, and endoparasitic nematode (Mermithidae) coupled with environmental stresses (physical factors) was recorded in suppressing the larval stages. In the same way, different duration of life cycles was observed with different species of beetles involved in different agro-ecological zones. The larval duration of *Lepidiota albistigma* Burmeister, was found significantly longer (284 days), followed by *Maladera affinis* Blanchard (58 days) and *Xylotrupes gideon* L. (57 days) and this parameter was found significantly different ($p<0.001$) among the species.

Population dynamics in Gunganagar (Chitwan) provided estimates of annual crop losses due to Scarabaeid larvae from 12-35% however, in epidemics this figure raises depending on the season and locality. Farmers may underestimate the role of these soil pests as only 16% of the framers (50 farmers household) surveyed in Gaindakot (Nawalparasi) site mentioned white grubs as a pest, whereas 90% did mention this as major pest in Chitwan. Collection of beetles from digging and light traps since 2003-2005 revealed a large number of beetle species in Nepal. The study so far indicated eighty seven different species of beetles in the study sites. The most important and frequently occurring beetle species were *Adoretus lasiopygus* Burmeister, *Anomala dimidiata* Hope, *Maladera affinis* Blanchard, *Heteronychus lioderes* Redtenbacher, *Anomala bilobata* Arrow, *Anomala xanthoptera* Blanchard, *Maladera cardoni* Brenske, *Idionychus excisa* Arrow, *Anomala cantori* Hope, *Mimela silguria* Arrow. Based on sampling studies and farmers observations, these species occur lesser or greater extent annually in all the study sites.

The population dynamics through digging indicates that a soil depth up to 20 cm is the most preferable depth for larval activity, whereas eggs are mostly laid up to 15 cm. The pupae and adults are concentrated somehow deeper than the other stages and were mostly found during the winter months. Larval activity in terai conditions was found highest during March-April and June-July. The possible reasons may be due to the availability of the host crops and favorable environment coupled with overlapping generations in the same environment. Similarly, the pupae and adults observed during winter months in the soil and only very few species such as *A. dimidiata* and few other congregate in tree plants for breeding purposes and majority of them held breeding unnoticing. The knowledge on the overall handling of the fungus, application, virulence, growth and survival of the biocontrol agents in the soil coupled with the pest identity and biology are important aspects for the development of biological control strategies in most of the environments.

## GLOSSARY OF TERMS USED

| | |
|---|---|
| Abdomen | The hindmost part of the three main body divisions of an insect |
| Cetoniinae | The subfamily of Scarabs which contains the flower beetles |
| Coleoptera | The name of the insect order that is the beetles |
| Coprinae | Beetle sub-family that are normally involved in dung and commonly known as dung beetle |
| Diapause | Hibernating stage of the beetles, normally occurs during pupal stage |
| Dorsal | Relating to the back or top side of an animal |
| Dynastinae | The subfamily of scarabs which contains the "rhinoceros" beetles |
| imago | The adult stage of an insect |
| Lamelliform | Composed of or furnished with lamellae. Said of the antennae of scarab beetles as they are of a manifold design which can be unfurled like the rays of a fan |
| Melolonthinae | The beetles sub-family normally occur during May -June and commonly known as cockchafer |
| Multivoltine | Beetle species with more than generation per year |
| Phototropic | Beetle species that are commonly attracted to light during night |
| Polyphagous | Organisms that eat all types of plant species |
| Pronotum | The dorsal surface or sclerite of a beetle's 1st thoracic segment |
| Rutelinae | The subfamily of scarabs which contains the "shining leaf" beetles |
| Subfamily | A sub-division of a family, containing genera which are visibly different enough in form to warrant a minor separation from the main family classification |
| Subspecies | A sub-division of a species, usually inhabiting a particular area, visibly different from other populations of the same species |
| Univoltine | Beetle species with one generation per year |
| White grubs | Larvae of insects of Scarabaeidae family of the order Coleoptera |
| Mermithidae | Endoparasitic nematodes also the pathogen of larval stage of the beetle |
| Microbial control | Insect pest control using microbial organisms |
| Biological control | Insect pest control with the use of natural enemies such as microbial agents, predators, parasitoids |
| Entomopathogens | Microbial pathogens that are pathogenic to insects |
| *M. anisopliae* | Insect pathogen that is commonly known as green muscardine fungus |
| *B. bassiana* | Insect pathogen that is commonly known as white muscardine fungus |
| Spores | The reproductive organs of a fungus |
| Conidiospores | The fungus spores produced in aerial environment i.e. outside the insect body cadavers |
| Blastospores | The fungus spores produced only in the heamolymph of the living insects and in submerged cultures |
| Sterile soil | Soil prepared after autoclavation at 120 $^{o}$C for one hour and cooled for bioassay experiments |
| Traditional farming | Farming with the use of local varieties and age old cultivation practices |
| Bariland | Un-irrigated upland area not suitable for rice cultivation |
| Khetland | Irrigated lowland area primarily meant for rice cultivation |
| Terai | Almost flat and low land area of Nepal |
| Tween 80 | Chemical used for making the hydrophobic fungus spores into hydrophilic |

## ABBREVIATIONS RELATED TO EXPERIMENTS

| | |
|---|---|
| µm | Micro milliliter |
| $^{o}C$ | Degree Celsius |
| ANOVA | Analysis of variance |
| ARS | Agriculture Research Station |
| asl | Altitude from Sea Level |
| B. b. | *Beauveria bassiana* |
| BCA | Biocontrol agents |
| BHC | Benzene hexachloride |
| Bt | *Bacillus thuringiensis* |
| CFU | Colony Forming Units |
| cm | Centimeter |
| CRD | Completely Randomized Design |
| CV | Coefficient of Variation |
| DADO | District Agriculture Development Office |
| DDT | Dichlor diphenyl trichlor ethane |
| d. f. | Degrees of freedom |
| DMRT | Duncan's Multiple Range Test |
| DOA | Department of Agriculture |
| DFID | Department for International Development Agency of the Government of United Kingdom |
| EPSF | Entomopathogenic Soil Fungi |
| $F_2$ generation | Second generation |
| FAL | Federal Research Station for Agriculture and Agroecology, Reckenholz, Zurich |
| FYM | Farm Yard Manure |
| g | Gram |
| $g^{-1}$ | Per gram |
| GBM | *Galleria* bait method |
| GENSTAT | Computer Software Packages for Statistical Analysis, Waterhouse, UK |
| h | Hours |
| ha | Hectare |
| IAAS | Institute of Agriculture and Animal Sciences |
| INGOs | International Non-Governmental Organization |
| IPM | Integrated Pest Management |
| kg | Kilo gram |
| km | Kilo metre |
| kg/ha | Kilo gram per hectare |
| lb | Pound |
| L | Litre |
| $LC_{50}$ | Which produces mortality in 50% of the test hosts |
| L1 | First instar larval stage |
| L2 | Second instar larval stage |
| L3 | Third instar larval stage |
| $LD_{50}$ | Lethal Dose 50 (Fifty percent death of the tested population from a particular dose of fungus strain) |

| | |
|---|---|
| LSD | Least Significant Difference |
| $LT_{50}$ | Lethal time 50 (Fifty percent mortality of the tested population within a particular time due to fungus strain) |
| m | Meter |
| M. a. | *Metarhizium anisopliae* |
| M. Sc. | Master of Science |
| $M^2$ | Meter square |
| $m^{-2}$ | Per meter square |
| MCAs | Microbial control agents |
| ml | Mililitre |
| Mm | Milimetre |
| MSTAT-C | Michigan State University Statistical Computer Software Package, USA |
| NARC | Nepal Agricultural Research Council |
| NGO | Non-Governmental Organization |
| NMRP | National Maize Research Program |
| ODA | Overseas Development Agency |
| NPV | Nuclear Polyhederosis Virus |
| ns | Non-Significant |
| p | Probability |
| P | Percentage |
| Ph.D. | Doctor of Philosophy |
| Pvt. organization | Private organization |
| RPM | Rotation per minute |
| r | Regression |
| s | Seconds |
| SDC | Swiss Agency for Development and Cooperation |
| RCBD | Randomized Complete Block Design |
| RH | Relative humidity |
| RONAST | Royal Nepal Academy of Science and Technology |
| Ropani | Unit of a area for the measurement of the land which is equal to 5000 square metre |
| SDA | Sabouraud Dextrose Agar |
| SEM | Standard error of the mean |
| SM | Selective medium |
| SSMP | Sustainable Soil Management Programme |
| TU | Tribhuvan University |
| t/ha | Ton per hectare |
| VDC | Village Development Committee |
| v/v | Volume by volume |
| UV ray | Ultra violet rays |
| WP | Wettable powder |
| % | Percentage |

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# Chapter 1

# General introduction and objectives

# Chapter 1: General introduction and objectives

## 1.1 STATEMENT OF THE PROBLEM

White grubs (Coleoptera: Scarabaeidae) are soil inhabiting and root feeding immature stages of scarab beetles. The white grub family, Scarabaeidae is second largest and omnipresent family within the order Coleoptera. The world fauna of white grub exceeds 30,000 species (Mittal, 2000) and there are about 1300 North American species (Borror *et al.* 1975). The maximum number occurs in the tropical areas of the world, particularly in African and Oriental regions. The fauna of the Indian sub region is very rich and diverse, but it is yet to be fully explored (Mishra and Singh, 1999). White grubs have become serious pests of most agricultural crops, fruits, vegetables, ornamental plants, plantation crops, pastures, turf and meadow grasses, lawns, golf courses and forest trees in different part of the world (Guppy and Harcourt, 1970; Potter *et al.* 1992). White grubs are polyphagous pests having a wide range of hosts, which are damaging both on adult and larval stages; however the larvae are a greater nuisance. Beetles are defoliating pests and damage a large number of fruit crops and forest trees as a result of feeding on apical buds and tender leaves, whereas, the grubs feed on plant roots, causing yellowing. They cause wilting which is characterized by an initial purpling of the leaves, followed by death of small plants and reduced vigor or lodging of larger ones. Sometimes affected plants produce dead hearts. In tuber and other underground crops larvae feed by making circular holes into them thus rendering them unfit for marketing. Some plants wilt and ultimately die; such plants can be easily pulled out. The extent of damage caused by white grubs solely depends upon the species involved, the numbers present and host crop. In India, white grub is one of the five pests of national importance (Yadava and Vijayavergia, 1994). In many crops, white grubs cause losses to the extent of 40-80 % (Prasad and Thakur, 1959).

With increasing population pressure, Nepalese farmers have intensified their land use over the past decades with high value cash crops such as potato, vegetable, ginger and coffee into their farmland. As a result of this intervention coupled with the use of haphazard chemical pesticides, the scarabs have become increasingly serious as agricultural pests. In Nepal, quantification of their damage has not been assessed (G.C. and Keller, 2002) and strategic management of the problem is lacking. They are basically managed with synthetic chemicals and traditional methods; however, novel pest control based on microbial pesticides has not been initiated in the country. There has been no comprehensive quantification of their importance in Nepal, however, collaborating institutions of the Sustainable Soil Management Programme (Helvetas/Nepal) have reported white grubs as major limiting factors to crop productivity and infestation is increasing every year in upland crops in Nepal (Thapa and G. C., 2000). For example, they are attributed to have caused an average of about 25% yield loss on groundnut (*Arachis hypogea)* in Western Nepal. Infestation is also reported by several INGOs/ NGOs, farming communities and Government organizations every year from different parts of the country (Anonymous, 1995). During the 2001 and 2002 field survey in the western hills of Nepal, farmers reported that pest infestation is increasing every year (Pers. comm. with farmer).

Several species of the beetles are known to be notorious pests, both at the larval and adult stages. Until recently three main genera such as *Phyllophaga* spp, *Holotricha* spp and *Anomala* spp were reported to be major pests in Nepal (Joshi, 1994; Neupane, 1995), however, there are numerous unknown species involved in damage to the agricultural crops. On the other hand, the biology of white grubs has been extensively studied in different countries and reviewed by Richter (1961) and keys for identification of the main groups are provided by Crowson (1954) and Endrodi (1985). However, the damaging species and the biology of the white grub species present in Nepalese agriculture have not been studied and the extent of their damage has not been assessed so far. Therefore, it is urgently needed to understand the biology of damaging species and associated crop losses before planning strategic management of their populations

Crop protection has been practised since quite a long time and it passed through different phases as per the prevailing situations and the available options (van Emden *et al.* 1996). In the present scenario, crop protection has undergone dramatic change in most parts of developed and developing countries. The emphasis has shifted from the hitherto dominant chemical pesticides to integrated pest management (IPM), where the focus is on biological control and other natural resources with reduced reliance on chemicals. Such a change became imminent mainly because of the increasing failures of chemical pesticides in controlling most of the major pests (and diseases) and also due to the ever-increasing global awareness about the undesirable side-effects of these deadly poisons such as environmental pollution, health hazards, destruction of beneficial organisms, pest resurgence, secondary pest outbreaks, biodiversity, deterioration of plant and soil health etc (Baker and Gyawali, 1994). Pesticides, once thought to be a panacea for all our pest problems, are today viewed with suspicion and great concern.

Modern approaches to crop protection rely on management rather than control or eradication. In this endeavour, a pest species is considered a pest only when its population reaches levels that can cause yield reduction. Natural factors - such as natural enemies - that prevent a pest species from increasing are emphasized. Pesticides are used only as a last resort to bring pest densities down when crop loss is expected to exceed the cost of treatment. Novel pest control emphasises the use of biological control and other control measures, and especially the chemicals must play a supportive, rather than disruptive role. Chemicals should not be used on a 'calendar' basis but strictly when needed as defined by systematic pest monitoring. Selective rather than broad-spectrum chemicals should take preference. The aim is to produce high-quality marketable produce at minimal cost by intelligently using the various control options to manage pests.

Historically many insect pests, including white grubs have been controlled using persistent chemicals, i.e. organochlorines such as chloradane, aldrin, dieldrin, heptachlor (Niemczyk and Lawrence, 1973); DDT, BHC, carbaryl, malathion endrin (Kaunsale *et al.* 1978); phorate, carbofuran, quinolphos (Misra, 1995), fenitrothion and diazinon (Fujiie and Yokoyama, 1996) were found ineffective in controlling white grubs. Use of carbamids and pyrethroids was also common. The larvae present in the soil do not come into direct contact with the insecticides (Wegner and Niemczyk, 1981). The insects have also shown resistance to many chemicals. The uses of chemical insecticides applied to the white grubs in the soil also have hazardous effects on some non-target soil organisms. Although, organophosphates are an improved form over organochlorides, non-target effects and health concerns still

prevail, which has led to renewed efforts in identifying alternative forms of control such as the use of microbial control, i.e. the use and action of enemies including pathogens etc. that have received considerations in different part of the world (Bednarek *et al.* 2000).

Several predators and parasitoids have been identified but their unsuitability for manipulating the process has led to the investigation of insect pathogens such as *Beauveria bassiana* (Balsm.) and *Metarhizium anisopliae* (Metsch.), which are considered to have a great potential. Several isolates of *M. anisopliae* have been identified to be highly virulent against the insects living in soil and cryptic habitats and have been subsequently used in research against a range of insect pests. Unlike agrochemicals, which are tested in the field very early in their development, microbial control agents (MCAs) tend to be developed in a laboratory and/ or glasshouse environment over a long period of time.

The potential of fungal entomopathogens for insect pest control was recognised at the time of the description of *B. bassiana*, and already in 1884 E. Metchnikoff had put in place a small plant for the production of fungal inoculums to control the sugar beet weevil. Research in the second half of the last century favoured the development of *B. thuringiensis*, which was the first microbial insecticide to obtain practical and economical significance. Microbial control strategies are valuable components in integrated pest management and have advantages over chemical pesticides (Rosset and Moore, 1997). Meanwhile, a number of fungal species have been investigated, undergone commercial development and reached registration. Products, based on *B. bassiana, B. brongniartii, M. anisopliae, Paecilomyces fumusoroseus* and *Lecanicillium lecanii* are applied in a number of countries namely in Europe (Switzerland, Austria) New Zealand and Australia (Keller, 2000; Rath *et al.* 1995). Microbial control is compatible with biological, toxicological, environmental and social requirements (Pereira and Roberts, 1991). The sustainability and economics of production of microbial agents is very important which also rests on the efficacy to the target organisms and at the same time to the non-target organisms (Burges and Hussey, 1971). The host range of *M. anisopliae* (Metsch) Sorokin is wide exceeding two hundred species of seven orders of the insects (Robertson, 1993). The pathogenicity however varies with strains or isolates (Aizawa, 1987). Therefore, the selection of effective strains of entomopathogens is essential for the development of microbial insecticides.

In general, a strategic implementation of microbial control includes several components that require an understanding of host insects and pathogens, and the behavior of the host and pathogenicity. At the same time, farmers' knowledge is of prime important for any scientific study (Gurung, 1985). The severity of white grubs' problem in Nepal is also due to the involvement of a range of species within the same area. The white grub species are both useful and harmful. The beneficial ones include the coprophagous or saprophytes which play a significant role in nutrient cycling as scavengers (Mittal and Vadhara, 1998). The others are harmful, as they are phytophagous and damage crop plant species (Arrow, 1917). Therefore, correct identification of a pest species and understanding of their life cycle is essential before initiating any control measures. Keeping these points in view, the present study is, therefore, focused on exploring the indigenous fungal antagonists in Nepal, method of production and use against damaging species of white grubs in particular and soil pests in general. The study is broadly divided into three parts, i) study on the host aspects, identification of damaging species of white grubs in the research command area of Nepal, ii) study on the indigenous species of entomopathogenic fungi, especially *M.*

*anisopliae* (Metsch.) Sorokin and, iii) assessment of microbial control into tiered experiments, i.e. both indoors and outdoors, and every area will include several sub-activities.

## 1.2 AIMS OF THE STUDY

The wider objective of the study is to know the fundamental knowledge of the insect-pathogenic fungus, *M. anisopliae* and to use that knowledge to contribute to ecological and economic sustainability of intensified agricultural production systems through effective control of soil insect pests to uplift the livelihood of hill farm families of Nepal.

The immediate objectives are:

1) To identify the damaging species of scarab beetles in the research sites of Nepal.
2) To understand the phenology of the commonly available beetle species in the research sites.
3) To explore and identify the indigenous species of insect fungi which are pathogenic to white grubs in the agricultural fields of Nepal.
4) To improve the virulence and efficacy of the entomopathogenic fungus, *M. anisopliae* through a tiered screening strategy.
5) To develop mass production methods with fungus isolates of *M. anisopliae* in different cereal substrates.
6) To evaluate the entomopathogenic fungus *M. anisopliae* against white grubs in farmers' fields.
7) To develop a suitable recommendation for eco-friendly control of white grubs to various counterparts in Nepal.

This work encompasses fundamental as well as applied field and laboratory research work based in Switzerland and Nepal. Laboratory research will be focused on factors that affect fungal viability, infectivity and insect susceptibility through bioassay to the white grubs. Virulent strains will be screened out and mass produced for field test against white grubs. Novel fungal strains will be assessed in terms of mycoses to the common white grubs' species of some agro-ecological zones of Nepal. Fungi used in this way will provide safe, effective biological alternatives to synthetic chemical insecticides.

## 1.3 THESIS ORGANISATION

This thesis is divided into twelve different chapters. Following the introductory chapter with thesis objectives and organization as described in Chapter 1, current knowledge on the insect pathogenic fungi and its possible integration into white grub control, with regard to the Nepalese situation, is reviewed in Chapter 2. This chapter is large and, for convenience, is divided into two sections explaining the general review of the literature on white grubs in section 1, and insect pathogenic fungi in Section 2. Materials and methods relevant to specific experiments are explained in the following respective chapters. Chapter 3 presents the exploratory and foundation works on which the further activities of the thesis are dependent. Preliminary screening of the *M. anisopliae* isolates was conducted and the results are presented in Chapter 4. Chapter 5 describes the pathogenicity of the isolates carried out into second tiered experiment with the dominant species of white grubs

abundant in different agro-ecological zones of Nepal. Efficacy of commercially available exotic strains of *M. anisopliae* and *B. bassiana* are assessed as with the indigenous strains and the findings are included in Chapter 6. Mass production of insect pathogenic fungi into suitable solid substrate for field application is explained and results are presented in Chapter 7. Chapter 8 presents the results of the efficacy of entomopathogenic fungus, *M. anisopliae* after application as bio-control agent against white grubs under field conditions in Nepal. Chapter 9 presents the results of monitoring studies of white grubs in different agro-ecological zones of Nepal. In this chapter, results of the most abundant species of white grubs with their identification are included. The results of the mortality factors and life cycle of the most common and damaging species are included in Chapter 10. In the same way, the population dynamics of the damaging species are established in the same sites where fungus applications are undertaken and the findings are included in Chapter 11. The conclusions of the overall findings from different experiments are in the Chapter 12.

Some chapters or part of the information of some of the chapters are presented in scientific publications and proceedings. The references related to individual sections and chapters are included at the end of each section and chapter. A table of contents for all chapters is presented at the beginning of each chapter. Appendices and tables are numbered corresponding with the number of each chapter. At the end, a short biographical sketch of the writer is presented.

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# Chapter 2: General review of the literatures

# Chapter 2: General review of the literatures

Several literatures suggest that many studies have been made on white grubs and its antagonist pathogen, *Metarhizium anisopliae* (Metsch.) Sorokin in different aspects in different parts of the world. In this thesis, attempts have been made to review the available literatures on both the fungus and host with especial emphasis of the possible exploitation of this pathogen for white grub management in Nepal.

## SECTION 2.1: THE INSECT PATHOGENIC FUNGI ATTACKING WHITE GRUBS

Microbial control to various pest insects has successfully been applied in other countries like Switzerland, Austria, New Zealand and Australia (Keller, 2000; Strasser, 1999). They were either based on the insect pathogenic bacteria *Serratia entomophila* (Jackson *et al.* 1992) or the insect pathogenic fungi, *Metarhizium anisopliae* (Zimmermann, 1993) and *Beauveria brongniartii* (Zimmermann, 1992) against wide range of soil insects and insect living in cryptic habitats, where health consideration is very important.

### 2.1.1 Historical aspect of microbial control

Biological control or bio- control, is defined as "the use of living organisms (and viruses) to suppress the population density or impact of specific pest organisms, making it less abundant or less damaging than it would otherwise be" (Eilenberg *et al.* 2001). Studies of natural epizootics of entomopathogenic fungi (Hyphomycetes or anamorphic fungi or microsporic fungi) during the latter half of the nineteenth century and the first half of the twentieth century. About the same time, mycologists were beginning to report the parasitization by fungi of insects other than the silkworm. Robin (1853), Fresenius (1858), Brefeld (1870), and Lohde (1872) were among those making the first scientific observations on entomogenous fungi. Then, Metschnikoff in 1879, in Russia, made the first significant experiments on the destruction of injurious insects by the use of microorganims when he was able to infect larvae of the beetle, *Anisopliae austriaca* (Hbst.) with the fungus *Metarhizium anisopliae* (Metsch.).

The use of micro-organisms such as bacteria, fungi, nematodes, virus, protozoa that can cause disease in insects have also reached commercial scale and some organisations have attracted a lot of interest due to their control potential and their ability to multiply in vitro. Several fungi such as *B. brongniartii, B. bassiana, M. anisopliae,* viruses such as nuclear polyhederosis virus (NPV), baculoviruses, and nematodes (Steinernema, and Heterorhabditis) are the major recent research focus. The biocotrol methods require detailed information about host, pathogen, and their dynamics such studies have been started in a wider range only recently (Allen, 1980). The major advantages of using microbial agents are narrow host range, high virulence, safety for non-target organisms, compatibility with some fungicides and many other types of pesticides, ease of production with local materials and application without using costly equipment.

### 2.1.2 Insect pathogenic fungi

Fungi are a phyllogenically diverse group of microorganisms that are all heterotrophic (absorptive nutrition) eukaryotes, unicellular (i.e. yeasts) or hyphal and reproduce by sexual and or asexual spores. The higher taxa of fungi have undergone drastic revision in recent years. The true fungi (Kingdom: Mycota) are divided into four divisions: the Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota (Hawksworth *et al.* 1995). Ainsworth (1971), separates the fungi into two major divisions, Myxomycota for plasmodial forms and Eumycota for nonplasmodial forms that are frequently mycelial. Although a variety of criteria are used, the most common character used to assign fungi to natural groups (i.e. based on genetic relationships) relies on sexual fruiting structures. However, a large group of fungi, including many entomopathogenic taxa, have lost the ability to produce or rarely produce sexual spores. This group of fungi was traditionally placed in the division, Deuteromycota, within the artificial class, Hyhomycetes, microsporic or anomorphic fungi (Inglis *et al.* 2001). Entomopathogenic fungi are found in the division Eumycota and in the following subdivisions: Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina. Most entomopathogenic fungi are in Zygomycotina, class Zygomycetes, order Entomophtharales; in Ascomycotina, class Pyrenomycetes, order Sphaeriales, ectoparasites and in the Deuteromycotina, class Hyphomycetes, order Moniliales. They are known to infect all life stages of insects and are commonly found in aquatic, terrestrial, and subterranean habitats (Ferron, 1978). There are no known entomopathogenic forms in the division Myxomycota (Tanada and Kaya, 1993).

### 2.1.3 Major characteristics of Hyphomycetes

The Hyphomycetes are a large, rather heterogeneous group of fungi which include *Aschersonia, Beauveria, Culicinomyces, Hirsutella, Metarhizium, Nomouraea, Paecilomyces, Tolypocladium* and *Verticillium* (Inglis *et al.* 2001). They are characterised by mycelial forms that bear asexual spores, termed “conidia” borne on specialised conidiogenous cells, lack of a teleomorph state (Samson *et al.* 1988; Humber, 1997), however, few taxa such as *Aschersonia* and *Sorosporella* rarely or never produce exogenous conidia (Inglis *et al.* 2001). Most entomopathogenic Hyphomycetes are obligate pathogens under natural conditions. Nevertheless they grow relatively easily in pure culture on defined or semi-defined media. The conidia are microscopic in nature and susceptible to adverse environmental conditions, desiccation and ultraviolet radiation. All entomopathogenic species with exception of a several Entomophthorales can be cultured on artificial media.

### 2.1.4 Major hosts of insect pathogenic fungi

Fungi belonging to the family Deuteromycetes and Entomopthora causes mycosis in insects. More than 800 fungal species comprising of 125 genera have been reported to infect insects. They are very specific to insects, often to particular species, and do not infect animals or plants. The most comprehensive list of hosts was provided by Veen (1968), who compiled records of 204 naturally infected insects. Most insect pests are susceptible to fungal pathogens. Some fungi, such as the *Entomophthora* and related species, are fairly specific with regard to the groups of insects affected; others, such as *Beauveria*, have a wider host range. Most of these hosts are soil-dwelling and include over 70 Scarabaeid

larvae. Fungi infect individuals in all orders of insects; most common are Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera, and Hymenoptera (David 1967). The major host insects infected with *M. anisopliae* is presented in Table 2.1.1.

*M. anisopliae* is being tested as a natural enemy of white grubs (scarabs), corn rootworm, and some root weevils. It has a very broad host range and is extensively used in different parts of the world which is presented in detailed in the Appendix 2.1.1.

Table 2.1.1 Common entomopathogenous fungi and their major hosts (Source: Butt and Goettel, 2000).

| **Entomogenous fungus** | **Invertebrate host** |
|---|---|
| **Division Zygomycotina** | |
| *Conidiobolus obscurus* | Aphids |
| *Entomophaga aulicae* | Lepidopteran insects |
| *Entomophaga grylli* | Orthopteran insects |
| *Entomophthora muscase* | Dipteran insects |
| *Entomophthora thripidum* | Thrips |
| *Erynia neoaphidis* | Aphids |
| *Massospora cicadina* | Cicada, aphids |
| *Neozygites fresenii* | Certain Hemiptera and Lepidoptera |
| *Zoophthora radicans* | Whiteflies, scales |
| **Division Deuteromycotina** | |
| *Aschersonia aleyrodis* | Wide host range |
| *Beauveria bassiana* | Cockchafers and borers |
| *Beauveria brongniartii* | Cockchafers |
| *Culicinomyces spp.* | Spider mites, citrus mites, mosquitoes |
| *Hirsutella thompsonii* | Mosquitoes |
| *Metarhizium album* | Orthopteran insects |
| *Metarhizium anisopliae* | Wide host range |
| *Metarhizium flavoviride* | Lepidoptera |
| *Nomuraea rileyi* | Coleoptera, Lepidoptera |
| *Paecilomyces farinosus* | Wide host range |
| *Paecilomyces fumosoroseus* | Mosquitoes |
| *Tolypocladium cylindrosporum* | Wide host range |
| *Verticillium lecanii* | Aphid |

### 2.1.5 Taxonomy of *Metarhizium anisopliae*

*Metarhizium* is one of the best known genera of entomopathogenic fungi, commonly known as “green muscardine fungus” due to the green colour of the sporulating colonies and is applied as spores or mycelia in various formulations. The fungus was first isolated from the wheat chafer *Anisoplia austriaca* by Metschnikoff in 1879 and named

*Entomophthora anisopliae*. He suggested its use as microbial agents against insect pests (Steinhaus, 1949). The genus *Metarhizium,* was established by Sorokin (1883) retaining the scientific ethics. It is an imperfect; entomopathogenic fungus found in soils throughout the world. It was first recognized as a biocontrol agent in the 1880's (Genthner, 1995). Since then, *M. anisopliae* has been described from different groups of insects such as Orthropetera, Dermaptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera, including at least seventy Scarabaeidae species (Latch, 1965). In spite of the wide host range the isolates of the fungi have a high degree of specificity (Tanada and Kaya, 1993). Four groups of insect pests (termites, locusts, spittlebugs and beetles) are targeted for control by *M. anisopliae* (Zimmermann, 1993). Several other species of *Metarhizium* have been described from insects, including *M. flavoviride* from weevils (Gams and Rozsypal, 1973), however, only *M. anisopliae* has been found attacking scarabs.

The current classification of *Metarhizium* is based on morphological characters and was reviewed by Tulloch (1976), who only accepted *M. flavoviridae* and *M. anisopliae,* the latter with the short spored (ranging up to 8 µm in length), var. *anisopliae* and the long-spored var. *majus* (10 µm up to 14 µm) (Driver *et al.* 2000). *M. album,* described from a leaf hopper in Sri Lanka by Petch (1931), was determined by Toulloch to be an immature specimen of *M. anisopliae.* Rombach *et al.* (1987) also described the morphological resemblance of *M. flavoviridae* var. *minus* to *M. album. M. album,* which had been regarded as a synonym of *M. anisopliae,* was restored as a separate species and described as a pathogen on plant-and leaf hoppers from rice (Rombach *et al.* 1987). They considered the primary taxonomic criteria for delimiting species to be the shapes of conidia and conidiogenous cells, presence or absence of a subhymenial zone and whether or not conidia adhere laterally to form prismatic columns. They gave only secondary taxonomic value to the colour of the mycelium and conidia, and suggested that conidial size is useful in delimiting species. Several species of *Metarhizium* were described prior to 1976, but Tulloch (1976) accepted only *M. anisopliae* and *M. flavoviride;* all other species were synonmized or treated as varieties (Robert and St. Leger, 2004). The separation of species strictly on morphology and sometimes colour proved unsatisfactory for the wide number of isolates held in fungus collections world-wide since 1976. There have been efforts to add a molecular level to the taxonomic studies, and identification methods based on physiology and/ or nucleic acid have been attempted in recent years (Bidochka *et al.* 1994; Bridge *et al.* 1993). The general classification of *M. anisopliae* is presented in Table 2.1.2.

Table 2.1.2 Classification of *Metarhizium anisopliae* (after Ainsworth, 1971).

| | |
|---|---|
| Kingdom | Fungi |
| Division | Eumycota |
| Subdivision | Deuteromycotina |
| Class | Hyphomycetes |
| Order | Hyphomycetales |
| Family | Moniliaceae |
| Genus | *Metarhizium* |
| Species | *anisopliae* (Metsch) |

### 2.1.6 Morphology of *Metarhizium anisopliae*

Tulloch (1976) studied a number of species in this genus where, *M. anisopliae* appears white when young but as the conidia mature turn to dark green. Similarly, *M. album* produces white colonies and *M. brunneum* produces yellow or brown colonies. *M. anisopliae* has two types, the short spored form *M. anisopliae* var. *anisopliae* (conidia 3.5-9.0 μm) and long spored *M. anisopliae var* majus (conidia 9.0-18 μm). The morphological characteristics of mycelia, conidia and conidiophores depend on different factors such as temperature, pH, nutrition, light humidity and age of isolate (Tulloch, 1976). *M. anisopliae* forms a loose or tough mycelial mat with cushions or areas of conidial structures. Conidiophores frequently unite into synnemata. The shape of phialide is cylindrical. Conidia are produced in chains; conidiophores closely packed in sporodochial structures, conidia in columns. The conidia are ellipsoid, ends rounded or one slightly truncate, colonies grayish yellow, green.

### 2.1.7 Mycotoxins from *Metarhizium anisopliae*

Toxins produced by *Metarhizium in vitro* and potentially present in biocontrol formulations and/ or in fungus-killed insects were examined as to safety to non-target organisms (Stressed *et al.* 2000), with the conclusion that *M. anisopliae* will not secrete copious metabolites into the environment and that the toxins do not pose a health risk. Fungi usually cause insect mortality either due to nutritional deficiency, invasion and destruction of tissues, and release of toxins (Ferron, 1978). The green muscardine fungus produces toxic proteolytic enzymes (Kucera, 1980). A number of secondary metabolites act as mycotoxins and are produced by entomopathogenic fungi. Cultures of *M. anisopliae* contain the cyclodepsipeptides, destruxins A, B, C, D, and E, and desmethyldestruxin B (Kodaira, 1961; Suzuki *et al.* 1966). Destruxins B has been considered as new generation insecticides. They cause titanic paralysis when inoculated into larvae of *Galleria mellonella* (Roberts 1966) and cause death. The destruxins are toxic to insects only by ingestation and not through the integument. Cytopathology occurs in the mid-gut cells with changes in the mitochondria and endoplasmic reticulum causing strongly pycnotic nuclei (Tanada and Kaya, 1993). The secondary metabolites (e.g. destruxin E) may act as immunosuppressor, inhibiting the cellular and/ or humoral-host defence response.

The destruxins have been heavily researched by insect pathologists, plant pathologists and microbiologists, particularly because of their usefulness in understanding disease (Roberts and St. Leger, 2004). They have also received considerable attention from biochemists and natural-products chemists because of their very interesting cyclic hexadepsipeptide structure and the question of their synthesis *in vitro* and *in vivo* by fungi. Chemical synthesis methods are described in detail by Pedras *et al.* (2002). In addition to destruxins, swainsonine, a sugar analogue indolizidine alkaloid molecule and other small molecules are larger compounds which have deleterious effects on insects as reported by Mazet *et al.* (1994).

### 2.1.8 Biology of *Metarhizium anisopliae*

The life-cycle of *M. anisopliae* comprises both a parasitic as well as a saprophytic phase. The parasitic phase begins after the contact with a potential host (adhesion and germination

of the spore on the insect's cuticle), penetration into the haemocoel and development of the fungus (germination of the host's cuticle), followed by a rapid proliferation of fungal cell which ultimately results in the death of the host (Al-Aidross and Roberts, 1978). The parasitic phase is also divided into the two steps such as events before penetration and after penetration. The former step involves adhesion on the host cuticle, recognition and germination of the conidia, formation of appressorium whereas the later involves penetration, multiplication and growth of the fungus which are described into following heads.

2.1.8.1 Adhesion

Adhesive processes have not yet been intensively studied in entomogenous fungi; however, both physical and chemical interactions are probably important (Fargues, 1984). Samson *et al.* (1988), reported electrostatic forces and molecular interactions may be involved in adhesion. Enhanced adhesion of *Metarhizium* spores occurred concomitantly with the secretion of a mucilaginous coat during conidial hydration (St. Leger *et al.* 1986). Gene determining specific adhesion by *Metarhizium* spores was linked to that for brown spore colour, but the molecular basis for such specific adhesions remains to be established. Conidia strongly adhere to insect cuticles, and their attachment to cuticles is thought to involve non-specific adhesion mechanisms mediated by the hydrophobicity of the conidial cell wall (Boucias *et al.* 1991).

2.1.8.2 Pre-penetration and germination

After the pathogen reaches and adheres to the host surface, it proceeds with a sequence of activities such as spore germination, hyphal growth and formation of infection structures that may be stimulated or inhibited by the potential host. Spore germination is highly dependent on moisture and probably requires free water (Kramer, 1980) but this requirement may be met by moisture conditions of the microclimate in the absence of measurable precipitation (Ben-Zev and Kenneth, 1980).

2.1.8.3 Recognition factors

Several recognition steps may occur before penetration of the host cuticle. There is circumstantial evidence that recognition in some specific host pathogen associations may involve the highly specific binding of lectins and haptens, either of which occur on the pathogen or host (Kerwin and Washino, 1986). Although there is no direct evidence for lectin mediation in insect hosts, lectin-binding seems to be an important recognition in fungal-nematode and fungal-fungal interactions (Nordbring-Hertz and Chet, 1986).

2.1.8.4 Penetration into hosts

Penetration of the cuticle is accomplished by the germ tube itself or by the formation of an appressorium which attaches to the cuticle and gives rise to a narrow penetration peg (Boucias and Pendland, 1982). Penetration is both a mechanical and an enzymatic process (McCoy *et al.* 1988). With the help of lipases, proteases and chitinases, the germination tube penetrates through the cuticle and epidermis of the insect towards the haemocoel (St. Leger, 1993). However, in some cases, the fungus may not be able to penetrate the cuticle.

Inglis *et al.* (2001) reported number of factors, such as inappropriate moisture and inhibitory factors, such as fatty acids or melanin, within the cuticle are responsible. Some reports suggest, fungal penetration also occur through body openings such as buccal cavity, spiracles, and other external openings of an insect (Schabel, 1976). Penetration by fungi through the alimentary tract has been observed in worker termite, *Reticulitermes* sp (Krewin and Petersen, 1997) and the spiracles and pores of sense organs (McCauley *et al.* 1968).

2.1.8.5 Mode of infection

Fungi have a unique mode of infection in contrast to bacteria, protozoa and viruses. They reach the haemocoel through the cuticle or possibly through the mouth parts (Inglis *et al.* 2001). Ingested fungal spores do not germinate in the gut and are voided in the faeces. Infection therefore results from the contact between virulent infectious inocula (conidia) and a susceptible insect cuticle which allows germination and penetration of the germ tubes through the integument which results in the spread of the pathogen through the host tissues (Kurisu and Manabe, 1978). The mode of penetration mainly depends on the property of the cuticle, its thickness, sclerotization, and the presence of antifungal and nutritional substances (Charnley, 1984). The newly moulted larva and the newly formed pupa are more susceptible to infection than those in which the cuticle has fully hardened (Fox, 1961). Studies have demonstrated that some fungal pathogens produce insecticidal toxins important in pathogenesis (Burges, 1981).

After the germinating hypha has penetrated the insect's integument and entered the haemocoel, it produces yeast like hyphal bodies, essentially blastospores that multiply by budding. In addition to hyphal bodies, hyphal strands and wall-less protoplasts may develop in the haemocoel. The dispersal throughout the haemocoel and tissue invasion varies with the fungal species. Some fungal species form both hyphal bodies and protoplasts depending on the nutritional environment (Tanada and Kaya, 1993). After crossing the integument (Kaaya and Munyinyi, 1995), the muscardine develops in the haemocoel in the presence of cellular defensive reactions of the host (Seryczynska and Bajan, 1975). True pathogens such as *Metarhizium* produce toxins which erode the granuloma and allow blastospores to invade the haemocoel. Hyphal bodies proliferate only just before death of the host. Suzuki *et al.* (1970), reported the role of several types of destruxins of *Metarhizium* in the insect body tissues. The life cycle of *M. anisopliae* is similar with that of *Beauveria brongniartii* as shown in the below mentioned Figure 2.1.1, however, *M. anisopliae* produces no aerial mycellium and has very short and arranged conidia as shown in the fingures in Chapter 3 (Figures 3.5 and 3.6).

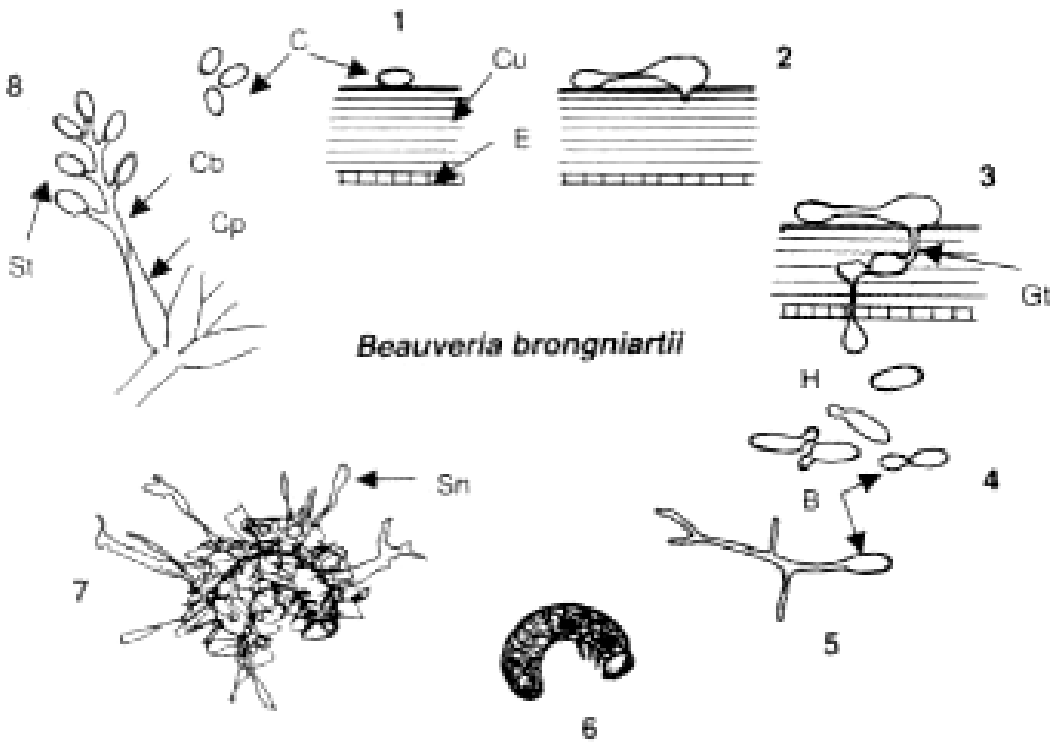


Figure 2.1.1 Life cycle of *Beauveria bassiana* comparing with *Metarhizium anisopliae* (1-5) parasitic and (6-8) saprophytic phase: (1) Adhesion of conidia (C) on the insect cuticle (Cu) (2) Germination of the conidia on the surface of the cuticle (3) Penetration of the germination tube (Gt) through cuticle and epidermis (E) into the haemocoel (H) of the insect (4) Production and multiplication of blastospores (B) in the haemocoel (5) Germination of blastospres and colonisation of the insect body (6) Mummified insect cadaver filled with mycelium of *B. bassiana* (7) Outgrowth of hyphal strands or *synnemata* (Sn) into the surrounding soil (8) Growth of conidiophores (Cp) with conidia forming cells (Cb) (Illustration by S. Keller, modified).

### 2.1.8.6 Signs and symptoms

At an early stage of fungal infection, the insect shows little or no signs and symptoms except for a few necrotic spots which may develop at the invasion sites. In advance stage of infection, the insects generally become less active, their appetites are reduced, and they lose coordination. Infected insects often move to high places or if subterranean, rise to the soil surface (McCoy *et al.* 1988). The time of tissue disintegration may differ with the fungal species, mode of invasion, and host species. The cells and tissues of an infected insect may begin to disintegrate prior to the insect's death (Lefebvre, 1934). The fungal hyphae continue to grow usually resulting in mummification, and the dead insects retain their form and shape. Shortly prior to or at death, the insect may have a characteristic colour.

### 2.1.8.7 Saprophytic development of the fungus

Host death marks the end of the parasitic phase of fungal development. In case of *Metarhizium* the insect cadaver looks white in the beginning and turns greenish black after full sporulation (end of saprophytic phase). The saprophytic phase commences when the infected insect dies and generally ends with the formation of reproductive organs. The saprophytic phase is essential for the completion of the developmental cycle of

entomogenous fungi. Reproductive spores are produced within the sclerotium or on sporophores (sporangiophore and conidiophore) (Ferron *et al.* 1991). Under unfavourable conditions, the fungus produces resistant or resting propagules such as chlamydospores, azygospores, zygospores, or oospores (Tanada and Kaya, 1993), however, hyphomycetes are unable to form resistant resting spores.

### 2.1.9 Different phases of fungal epiziotiology to insects

The principles of epizootics is extensively summarised by Keller and Zimmermann (1989) which is presented in Table 2.1.3.

Table 2.1.3 Characterisation of the different epizootiological phases.

| Epizootiological phases | Host population | Fungus population | Disease incidence |
|---|---|---|---|
| 1. Latent | Healthy or:<br>Disease not detectable:<br>density too low<br>sufficient defence reaction<br>Suffering from sporadic infection<br>restricted to single individuals<br>Population development not affected | Absent or:<br>Unable to cause infection:<br>Density too low<br>Low pathogenicity<br>Low density<br>Limited distribution<br>Low pathogenicity | Zero<br><br><br><br>Very low |
| 2. Enzootic (Pre-epizootic) | Small numbers of diseased individuals always present, distributed locally or throughout the population<br>Population development affected<br>Density tends to stabilize | Low or moderate density<br>Wide or overall distribution<br>Inducing low to moderate infection pressure | Low to moderate |
| 3. Epizootic | High proportion of diseased individuals distributed throughout population<br>Population development severely affected<br>Density decreases | High density, increasing<br>Overall distribution<br>Inducing high infection pressure | High |
| 4. Enzootic (Post-epizootic) | Moderate proportion of diseased individuals, decreasing, distributed throughout population<br>Population tends to stabilise at low density | High density, decreasing<br>Overall distribution<br>Inducing high to moderate infection pressure, decreasing | Moderate to low |

### 2.1.10 Factors affecting epizootiology

Natural disease development and spread are governed by host, pathogen, environment and humans and their interactions have been represented by the disease tetrahedron (Zadoks and Schein, 1979). These factors are interrelated and interactions are critical to overall system behaviour.

2.1.10.1 The insect host

One of the most important paradigms in microbial control is that “stressed” animals are more susceptible to entomopathogens than non-stressed animals (Vago, 1963). Variety of factors such as crowding, nutrition, exposures to chemical stressors, environments etc. is the major causal factors to stress the insects. Inadequate nutrition often leads to increased susceptibility to entomopathogens, and the utilisation of resistant plant genotypes to induce nutritional stress can also decrease the susceptibility of insect pests to entomopathogens. Conversely, diet can also increase the susceptibility of insect pests to entomopathogenic hyphomycetes (Ekesi *et al.* 2000).

Ferron (1981) reported that young larvae of the European corn-borer (*Ostrinia nubilalis*) are more susceptible to *B. bassiana* than older larvae. In contrast, adult western flower thrips (*Frankliniella occidentalis*) were more susceptible to *Verticillium lecanii* than larvae (Vestergaard *et al.* 1995). Similar result is suggested by Keller and Zimmermann (1989) that the second and third instars larvae of *Melolontha melolontha* are more sensitive to *B. brongniartii*. Vey and Fargues (1977), reported that infection rate is more advanced at ecdysis since the new cuticle is wounded during ecdysis because of adhesion of the two cuticles by mycelium.

2.1.10.2 The pathogen population

Properties of the pathogen population that are important in epizootiology include virulence and pathogenicity, dispersal and survival in the host’s environment, and inoculums density and spatial distribution. With a greater number of susceptible hosts, there may be a greater reservoir of inoculums available to produce an epizootic (Tanada, 1963).

2.1.10.3 Environmental factors influencing *Metarhizium anisopliae*

Environmental factors have dramatic effects on the efficacy of entomopathogenic fungi on insect pests. The most important abiotic factors are temperature, water availability, precipitation, and oxygen in the soil, pH, soil texture and wind (Zimmermann, 1982).

*Solar radiation*

Conidia may be very sensitive to solar radiation (Ignoffo *et al.* 1977), and spore longevity and germination may be improved if the microhabitat is suitable e.g. a dense crop canopy can protect the conidia from direct radiation. Under field condition, this may have marked effect when the solar spectrum is about 85-315 nm, therefore several oil soluble sunscreens and shade significantly increase the survival of *M. anisopliae* (Zimmermann, 1982).

*Soil temperature*

Goettel and Inglis (1997) reviewed the effect of temperature extensively. Most of the entomopathogenic fungi have a wide range of temperature tolerances (0-40 $^{o}$C), however, temperature optima for infection, growth and sporulation are usually much more restricted (generally 20-30 $^{o}$C). Propagules for most species survive well at sub-zero temperatures and can be stored for long periods at -20 $^{o}$C or in liquid nitrogen (-196 $^{o}$C). Spores of some

species can tolerate very high temperatures for very short periods (150 $^{o}$C for 30 seconds), however, the maximum threshold for long periods is usually close to 40 $^{o}$C (Goettel *et al.* 2000). The optimum temperature for *M. anisopliae* infecting adult thrips is 23 $^{o}$C (Vestergaard *et al.* 1995), and a decrease in temperature of 3-5 $^{o}$C increases the time to death by 1 day. In general, the optimum temperature for most entomopathogenic hyphomycetes is between 20 and 25 $^{o}$C but infection and disease can occur at temperatures ranging between 15 and 30 $^{o}$C. Above 30 $^{o}$C, the vegetative growth of most taxa is inhibited and growth usually ceases at 37 $^{o}$C. Most fungi are unable to germinate below 10 $^{o}$C or above 35 $^{o}$C. Inglis *et al.* (2001) reported that moisture can have very significant effects on the persistence of fungal inocula. For the most part, fungal conidia usually exhibit greatest stability under cool and dry conditions (Roberts and Campbell, 1977). In contrast, conidia of *M. anisopliae* survive better at moderate temperatures when relative humidity is high (Daoust and Roberts, 1983).

In tropical and sub-tropical regions, temperatures in the upper soil layer may reach 30 and 40 $^{o}$C. Temperatures as high as 70 $^{o}$C occurs commonly in compost heaps, an ideal breeding place for *Oryctes rhinoceros* whose larvae are often attacked by *M. anisopliae* (Johnpulle, 1938). Soil temperature should be taken into full account while applying the fungus in Nepal especially during summer season maize. According to the environmental conditions isolates with lower or higher temperature requirements can be selected (Latch and Kain, 1983).

*Relative humidity*

Relative humidity can influence fungal efficacy in several ways. Humidity, in combination with temperature, influences evaporation of spray droplets, which can result in the loss of small particles and thereby adversely affect targeting (Inglis *et al.* 2001). Furthermore, moisture can also have very significant effects on the persistence of fungal inocula. For the germination of infective units, a humid atmosphere usually in excess of 80% relative humidity is essential. Walstad *et al.* (1970) mentioned the limit between 92.3-100% for the best germination of the spores and below 90% sporulation is inhibited.

*Soil moisture*

Peak in vitro germination of *M. anisopliae* occurs at 25- 30 $^{o}$ C, with a germination range of 15-35 $^{o}$ C. Sporulation occurs between 10 $^{o}$C and 35 $^{o}$C. The thermal death point for fungi is approximately 50 $^{o}$C. Conidia are reported to survive more than a year at 8 $^{o}$C, but at 21 $^{o}$C, *B. bassiana* for 0.5 months and *M. anisopliae* for 2.5 months (Walstad *et al.* 1970).

*Rainfall*

In addition to increasing humidity, rainfall can serve to disperse conidia from substrates as well as aid in the dispersion of propagules. The effects of precipitation and/or dew on the foliar persistence of entomopathogenic viruses and bacteria have been relatively extensively studied (Griffin, 1963). In contrast, little is known about the persistence of entomopathogenic fungal propagules on insects and on foliage. Keller and Zimmermann (1989), reported that the rain has a great influence on the vertical movement of entomopathogenic fungi. Recent evidence indicates that rain causes significant removal of

*B. bassiana* and *M. anisopliae* conidia from foliage of monocotyledonous and dicotyledonous plants and from the integument of Colorado potato beetle larvae (*Leptinotarsa decemlineata)* (Inglis *et al.* 2000). Inyang *et al.* (2000), indicated that *M. anisopliae* is readily dispersed by rain splash in a rain tower experiment.

*Soil factors*

The soil is an excellent habitat for microorganisms and arthropods. Many economically important insects spend some portion of their life in the soil, and often these stages are destructive to crops (Villani and Wright, 1990). In addition to physical factors (e.g., soil texture and structure, soil water status, gases, temperature, pH), numerous biotic factors (e.g., host population, host plant of target insect, predators and antagonists of the biological control agents, alternate hosts) influence both infection and spread of disease in insect populations (Groden and Lockwood, 1991).

Clay particles are colloidal in nature and may adsorb mircoorganisms, so restricting their distribution and movement. They may also function as a protectant against biodeterioration and thus may increase the stability and the longevity of conidia or blastospores (Fargues, 1984). Soil water not only affects the growth and survival of microorganisms and insects, but also profoundly affects their movement (Keller and Zimmermann, 1989).

Oxygen percentage in soil decreases with depth and the rate of decrease is more rapid in clayey or silty soils than in sandy soils (Brady, 1974). Relative humidity above 92.5 and 85% are needed for spore germination, mycelial growth, and sporulation of *Beauveria* and *Metarhizium*, respectively (Walstad *et al.* 1970). A decrease in conidial survival with increased moisture may be a result of reduced oxygen, although oxygen and carbon dioxide concentration may have little effect on conidia as long as they are dormant (Ligg and Donaldson, 1981).

The soil microflora is also highly influenced by the soil pH. In general, high acidity decreases the growth of bacteria and increases that of soil fungi. In contrast, alkaline soils are more suitable for bacteria. Fungi are also more tolerant of very acid conditions and several species can grow even at pH 3 (Keller and Zimmermann, 1989). Fungistatic effect on germination of *B. bassiana* conidia increased exponentially with increase in pH from 5.1 to 7 (Groden and Lockwood, 1991).

2.1.10.4 Agricultural practices

Agricultural practices such as ploughing, crop rotation and application of pesticides and fertilisers may radically alter the population of soil organisms. This may be due to desiccation, a change in aeration of soil, a direct effect of pesticides on target or non-target organisms, or by the addition of un-decomposed or partially decomposed organic materials (Keller and Zimmermann, 1989).

2.1.10.5 Fungus persistence and dispersal

Persistence and efficacy of fungal propagules are affected by different factors in the soil environment such as by soil type (Storey and Gardner, 1988), moisture conditions (Studdert

*et al.* 1990), temperature (Ligg and Donaldson, 1981), pH, organic matter content and level of conductivity and antagonistic organisms (Fargues *et al.* 1983). The persistence of *M. anisopliae* in soil appears to have attracted little attention (Zimmermann, 1992), even though it is well adapted to tolerate the conditions in the soil.

Dispersal is necessary for the spread of the disease. Pathogen population density and spatial distribution are key factors in the development of an epizootic, as they affect the likelihood to contact viable hosts (Tanada and Fuxa, 1987). Small soil arthropods, especially mites and Collembola, may be very important in distributing entomopathogenic fungi (Keller and Zimmermann, 1989). They have further reported that Acari and Collembola can transport conidia of *M. anisopliae* passively through a vertical soil layer of about 15 mm. Some fungal pathogens cause their hosts to climb to aerial locations just prior to death (MacLeod, 1963). Such behaviour may aid dispersal of infective conidia which may rain down or blow to nearby hosts.

### 2.1.11 Handling of insect pathogenic fungi

Details on handling and diagnosis of diseased insects have been recently reviewed by Lacey and Brooks (1997). Production of fungus involves several steps starting with the isolation and ending with on suitable solid substrates. These steps are individually involved in the whole process of fungus production are as follows.

#### 2.1.11.1 Isolation of entomopathogenic fungi

Selective media rich with antibiotics and growth substances are frequently used for the isolation of entomopathogens (Goettel and Inglis, 1997). Entomopathogenic Hyphomycetes may be isolated directly from insect cadavers on which the fungus has already sporulated or from soils. Most common methods include the soil dilution plating (Beilharz *et al.* 1982) and insect baiting (Zimmermann, 1986). Insect pathogenic fungi can be baited using a variety of substrates such as hemp seed (Kerwin and Petersen, 1997), however, *Galleria mellonella* are normally used for this purposes. Using the soil isolation method, it is often difficult to detect spore numbers below $10^3$ conidia /g in soils, and in some cases relatively few isolates recovered are pathogenic to insects from the same habitat (Milner and Lutto, 1976).

#### 2.1.11.2 Production of fungal propagules

Laboratory-scale of production of entomopathogenic fungi are extensively reviewed by Goettel and Inglis (1997) and Papierok and Hajek (1997). More recent general reviews on mass production and formulation are those of Jenkins and Goettel (1997). The isolates can be stored at the suitable temperature usually at 22-25 $^oC$. Individual colonies free of bacteria can then be harvested using Tween 80 (0.2%). Continued subculturing can result in changes in virulence and other characteristics (Glare, 1992).

Submerged fermentation can be used for production of blastosproes and conidia (Ignoffo, 1981). Blastospores are produced relatively in a large quantities using liquid medium. Normal water, corn steep, potassium sulphate ($KH_2$ $PO_4$) and sodium phosphate ($Na_2$ $HPO^4$) are mostly used to prepare liquid cultures (Goettel and Inglis, 1997). Most often

blastospores are spherical, oval or rod-shaped single cells which usually germinate within 2-6 h. The medium used for this type of culture is presented in Table 2.1.4.

Table 2.1.4 Liquid medium for the mass production of *Metarhizium anisopliae* blastospores in a fermentor (Source: Goettel and Inglis, 1997).

| | |
|---|---|
| Corn steep | 40 g |
| $KH_2PO_4$ | 4.5 g |
| $Na_2 HPO_4$ | 7.6 g |
| Normal tap water | 1000 ml |

2.1.11.3 Enumeration of the propagules

The conidia of *M. anisopliae* are hydrophobic. Therefore, in order to make them hydrophilic they are usually suspended with a wetting agent such as Tween 80. Thoma haemocytometer is commonly used to quantify numbers of propagules per unit volume. The haemocytometer has two types of squares such as big and small and the big squares are further divided into 16 small squares and such 4 big squares are covered by double lines. Numbers of propagules are counted diagonally from each 4 big squares of both the chambers. The average number of propagules per cell are multiplied by volume conversion factor (e.g. as per the given calibration in haemocytometer) to obtain an estimation of the number of propagules per ml. When the concentration of propagules in the original suspension is too high to get an accurate count on the haemocytometer (e.g.> 300/cell), it is necessary first to dilute the suspension prior to enumeration (Goettel and Inglis, 1997).

2.1.11.4 Storage of the propagule

Once the fungus is cultured, it must be stored unless it is used immediately. Conidia and mycelium should be stored in cryovials under nitrogen, or freeze-dried and stored in sterile glass ampoules (Humber, 1997) for short or long term storage. Freshly harvested conidia can also be air dried and stored in desiccators at 4 $^{o}$C or room temperature. Several hyphomycete fungi such as *V. lecanii or M. anisopliae* can be stored as conidia bound to silica gel at -40 $^{o}$C (Hedgecoc *et al.* 1995). Most details on isolation and storage of entomopathogenic fungi are given by Goettel and Inglis (1997) and Papierok and Hajek (1997).

2.1.11.5 Bioassay and infectivity assessment

The biological assay or bioassay is a form of experiment for the estimation of the potency of a substance or comparing the efficacy of two or more substances by means of reaction that follows their application to living matter (Rangaswamy, 1995). In Entomology, the subject may be an insect, stimulus may be a pesticide or a microbial agent and response may be change in characteristics of insects in terms of weight, shape, size, appetite or death or behavioural changes. Virulence may be measured in a bioassay by exposing a known number of hosts to a known number of pathogen and observing the number of dead over time (Reichelderfer, 1993). In case of white grubs, they are kept under controlled conditions and the mortality is assessed for periods of more than two months depending on the life span (Glare and Milner, 1991).

The amount of microbial agent that is required to produce a desired mortality is a function of susceptibility of the host and virulence of pathogen (Steinhaus, 1949). The bioassay method should expose the pathogen to the host by the natural route (Reichelderfer, 1993). Insect cuticle is the site of infection of white grubs by fungi (Inglis *et al.* 2001). The dipping method is the most common bioassay method for the evaluation of fungus in white grubs (Keller, 2000). Susceptibility is often a quantitative phenomenon (Zimmermann, 1993). The high dosage of $10^8$ to $10^9$ conidia per ml causes mortality of 90% (Glare and Milner, 1991). The most pathogenic strains under optimal condition give an $LD_{50}$ of about $10^4$ conidia per ml. The $LC_{50}$ value 7 days after exposure to *M. anisopliae* was 0.7 mg of conidia per 100 gm adults at 22-24 $^oC$. A sharp increase in mortality was observed 3 days after treatment with 10 mg of *M. anisopliae* per 100 adults. The $LT_{50}$ value at 10 mg spores per beetles was 4.2 days (Lacey *et al.* 1997). Onset of mortality was delayed at lower doses.

### 2.1.12 Mass production of entomopathogenic fungi

Petri dishes and autoclavable plastic bags are recommended for small and larger-scale production, respectively. However, other containers such as pans, glass bottles and inflated plastic tubes have been used (Jenkins and Thomas, 1996). The production methods of some important fungi are summarised below (Table 2.1.5).

Table 2.1.5 Production and storage information on selected entomogenous fungi (Source: Butt and Goettel, 2000). * 1: Surface or submerged culture; 2: Live host; 3: Semi-solid or diphasic culture, ** C: Conidia; DM: Dry mycelium; B: Blastospores; Z: Zoospores; S: Sporangia, ***Sabouraud dextrose, egg yolk and milk agar

| Pathogen | Production Method * | Media | Form of inoculums** |
|---|---|---|---|
| *Aschersonia aleyrodis* | 1,3 | Chopped millet | DM, B, C |
| *Beauveria bassiana* | 1, 3 | Most nutrient agar and liquid media | DM, B, C |
| *Beauveria brongniartii* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Coelomomyces* spp | 2 | Host rearing medium | S, infected copepods |
| *Culicinomyces clavisporus* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Entomophaga* spp. | 1,2 | Sabouraud dextrose, egg yolk, milk agar | C |
| *Erynia neoaphidis* | 1,2,3 | SEMA*** | DM, C |
| *Hirsutella spp.* | 1,3 | Most nutrient agar liquid media | Submerged DM, C |
| *Lagenidium giganteum* | 1 | Different soil or liquid media | Submerged C, B, C |
| *Metarhizium anisopliae* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Nomuraea rileyi* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Paecilomyces farinosus* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Tolypocladium* spp | 1,3 | Most nutrient agar and liquid media | DM, B,C |
| *Verticillium lecanii* | 1,3 | Most nutrient agar and liquid media | DM, B, C |
| *Zoophthora radicans* | 1,2,3 | SEMA | DM, C |

Alternatively, cheaper substrates such as rice or shelled barley can be used in autoclavable bags or other containers especially when large amounts of inoculums are required (Aregger, 1992). Once the fungus has sporulated, conidia are harvested either by washing off using buffered water direct scrapping from the substrate surface (e.g. agar), or by sieving (e.g. rice). Mass production of *B. brongniartii* has good success using barley kernels as

blastospores (Keller *et al.* 2002). They have reported that barley kernel produces an average of 3.3 x $10^8$ conidia on the surface of the soil.

The application of 40 kg fungus kernels corresponds to a dose of 3.7 x $10^{14}$ conidia per ha or about 3 x$10^5$ conidia per g soil (Keller *et al.* 2002). Rath and Pearn (1990) also have summarised the method for developing mass production system utilising local cereal grains (Moscadi, 1988). *M. anisopliae* is grown on a large scale in similar to that used in the production of *Bacillius thuringiensis* and the spores can then be formulated as a dust. The fungal spores can also be grown on sterilized rice in plastic bags for small-scale production (Aquino, 1974). Other techniques of mass production are also used in Brazil; either in autoclavable plastic bags on a standard nutritive medium, e.g. dextrose agar (Moura-Costa *et al.* 1974).

### 2.1.13 Commercial development of mycoinsecticides

The number of products available and under development for the biological control of pests is summerised by Feng *et al.* (1994) and Butt *et al.* (2001) is presented in Tables (2.1.6).

Table 2.1.6 Mycopesticide products registered or under commercial development for microbial control of various insect pests. (Source: Wraight *et al.* (2001). (Conidia refer to aerial conidia unless otherwise indicated. AS = aqueous suspensions; ES = oil based emulsifiable suspension; G = granule; OF = oil flowable, WC = whole culture; WDG = water-dispersible granular; WP = wettable powder).

| Pathogen | Product trade name | Company/ government | Active ingredient | Formulation | Principle target pests |
|---|---|---|---|---|---|
| *B. bassiana* | Bea-Sin | Agrobiologicos del Nordeste (Agrobionsa) | Conidia | WP | Pepper weevil, boll weevil, whiteflies |
| | Boverin | Mexico<br>USSR (former)<br>Czechoslovakia | Aerial or submerged conidia and/ or lastosproes | WP | Colorado potato beetle, codling moth |
| | Boverol-spofa | Hoechst Schering | | WP | Colorado potato beetle |
| | Conidia | AgrEvo, Colombia | Conidia | WDG | Coffee-berry borer |
| | Mycrotrol/ Botani Gard | Mycotech, USA | Conidia | WP, ES, OF | Whiteflies, aphids, diamondback moth, thrips, grasshoppers |
| | Naturalis-L | Troy Bioscience, USA | Conidia | ES | Cotton pests |
| | Ostrinil | (Protioagro)Venezuela | Conidia | G | European corn borer |
| | Proecol | Mycotech, USA | Conidia | | Army worms |
| | CornGuard | Mycotech, USA | | | European corn borer |
| | Mycotrol GH | Czechoslovakia | | | Corn borer<br>Grasshoppers, locusts<br>Colorado beetle |

| | | | | | |
|---|---|---|---|---|---|
| *Beauveria* spp | Boverosil Engerlingspilz | Eric Schweizer, Switzerland | | | Cockchafers |
| *B. brongniartii* | Schweizer | Eric Schweizer Seeds. Switzerland | Conidia | G/WC | Cockchafer |
| | Engerlingspilz | Andermatt Biocontrol AG | Conidia | G | Cockchafer |
| | | Kwizda, Austria | Conidia | WC | Cockchafers |
| | Melocont | NPP, France | Conidia | G/WC | Cerambycid beetles |
| | Betel | | | | |
| *M. anisopliae* | Bio-Blast | EcoScience, USA | Conidia | WP | Termites |
| | BioGreen | Bio-Care Technology, Australia | Conidia | G | Red-headed cockchafers |
| | Name | EcoScience, USA | Conidia | WC | Cockroaches |
| | Bio-Path | Bayer AG, Germany | Mycellium | G | Black vine weevil |
| | BIO 1020 | Probioagro, Venezuela | Conidia | WP | Sugar cane spittlebug |
| | Cobican | PlanTerra-Produtos | Conidia | WP | Pasture spittlebug |
| | Metabiol | Brazil | Conidia | G/WC | Sugarcane spittlebug |
| | *Metarhizium* Schweizer | Eric Schweizer Seeds Switzerland | Conidia | WP | White grubs |
| | Metarril | PlanTerra, Brazil | Conidia | WP | Pepper weevil, boll weevil, |
| | Meta-Sin | Agrobionsa, Mexico | Conidia | WP | Sugarcane borer |
| | Metaquino | Brazil | | | Spittlebugs |
| *M. flavoviridae* | Green muscle | CABI BioScience, UK | | | Locusts, grasshoppers |
| *P. fumosoroseus* | Bemisin | Probioagro, Venezuela | Conidia | WP | Whiteflies |
| | Pae-Sin | Agrobionsa, Mexico | Conidia | WP | Whiteflies |
| | PreFeRal | Thermo Trilogy, USA | Blastospores | WDG | Whiteflies |
| | PFR 97 | Eco-tek, USA | | | Whiteflies |
| *V. lecanii* | Mycotal | Koppert Biological Systems, the Netherlands | Conidia | WP | Whiteflies Thrips |
| | Vertalec | Koppert, the Netherlands | Blastospores | WP | Aphids |

The research, development and final commercialisation of fungal biological control agents (BCAs) continue to confront a number of obstacles (Butt *et al.* 2001). However, there is

increasing interest in the exploitation of fungi for the control of invertebrate pests, weeds and diseases.

### 2.1.14 Formulation of entomopathogenic fungi

To maintain or improve the efficacy of the spores, different components can be added such as carrier, diluents, binder, dispersant, UV protectants and virulence-enhancing factors (Moore and Caudwell, 1997). The most widely used carriers are oil and water. Oils are reasonably effective in sticking spores to insect and plant surfaces and as protectant against desiccation (Inglis *et al.* 1996). In contrast, surfactants (e.g. Tween) need to be added to water to ensure conidial suspension, but these are toxic to conidia if used at high concentrations (e.g.>0.1% v/v). Incorporation of humectants (e. g. Silwet) and ultra violet blockers (e.g. Tinopal) can offer some protection against harmful UV radiation.

### 2.1.15 Storage of fungus materials

Once the fungus is cultured, it must be stored unless it is used immediately. Many fungi, especially from the Hyphomycetes, can be maintained *in vitro* on several media. Most of the methods used for preservation of fungal cultures can also be used for short or long term storage of fungal propagules. Conidia and mycelium should be stored in cryovials under nitrogen, or freeze-dried and stored in sterile glass ampoules for short or long term storage. Freshly harvested conidia can also be air dried and stored in a desiccator at 4 $^{o}$C or room temperature. Several hyphomycete fungi such as *V. lecanii*, *M. anisopliae* can be stored as conidia bound to silica gel at -40 $^{o}$C (Humber, 1997). It is well documented, however, that storage at refrigerator temperatures (approximately 4 $^{o}$C) affords much longer survival than higher temperatures, and that temperatures above normal room temperature can be severely debilitating (Robert and St. Leger, 2004). Where possible, oxygen in the package should be near zero (Milner, 1995) and RH below 8% (Daoust and Roberts, 1983). Packaging is frequently in polyethylene bags. *M. anisopliae* conidia prepared by EcoScience Corp. (Jin *et al.* 1999), and held at room temperature for two years and then at 4 $^{o}$C for five more years had a germination rate of over 80% (Robert and St. Leger, 2004).

In general, spore viability falls off rapidly at higher temperatures which are shown in Table 2.1.7.

Table 2.1.7 Effect of storage temperature on *Metarhizium anisopliae* DAT F-001 spore viability and predicted $LT_{50}$ (Source: Rath 1992).

| Storage temperature ($^{o}$C) | % viability after 12 weeks | Predicated $LT_{50}$ 10 $^{o}$C | 16 $^{o}$C |
|---|---|---|---|
| 5 | 100 | 137 | 65 |
| 10 | 100 | 137 | 65 |
| 15 | 50 | 147 | 70 |
| 20 | 13.5 | 168 | 79 |

Moisture content can be critical if cooling is not possible as reported by Hedgecoc *et al*. (1995). They found gradual decline of viability of *M. flavoviride* conidia with 5% moisture content, after 4 months storage at 38 $^{o}$C whereas a rapid loss of viability occurred at 15%

moisture content. Most details on isolation and storage of entomopathogenic fungi are given by Goettel and Inglis (1997), Humber (1997), Kerwin and Petersen (1997) and Papierok and Hajek (1997). The shelf-life of the formulates of the entomopathogenic fungi is at least twelve weeks at temperature of 10 $^{o}$C or less. The storage time of dry *M. anisopliae* conidia has been extended from a few weeks in early experiments to four years at 10 $^{o}$C or one year at 30 $^{o}$C (Jenkins *et al.* 1998).

Rath (1992), further suggested the major disadvantage of this formulation is the considerable storage, handling, and transportation costs associated with a 10% a. i. product. There is scope to reduce this by coating pure *M. anisopliae* spores onto pasture seeds in a manner identical to the inoculation of clover (*Trifolium* spp) with nitrogen fixing bacteria (*Rhizobium* spp).

### 2.1.16 Application and compatibility of biopesticides

The application method largely depends on the targets and mode of formulation. The planned application technology, of course, dictates many of the parameters of the final formulation. For example, applications of dust are very seldom done with entomopathogenic fungi, with the exception of control of stored-products pests (Lord, 2001). Clays, however, are used as diluents and desiccants in shelf packs of conidia (Moore and Caudwell, 1997). The hydrophobic conidia of *Metarhizium* mix very well in oils, and oils of many types have been used as bases for fungal products (Fargues *et al.* 1997). Although oils do not completely prevent wash-off from leaves by rain (Inyang *et al.* 2000), a number of vegetable oils and commercially available formulations designed for insecticide application were found to more evenly spread *Metarhizium* formulations over leaves than water formulations (Ibrahim *et al.* 1999). This spread is particularly important in the case of sessile insects such as immature whiteflies. Soil inhibiting insects such as white grubs are infested using granular form of mycopesticides. Mycopesticide formulated into granular form may be applied directly into the soil with the help of tractors such as in Switzerland and other developed farming whereas; bullock drawn plough may be used in case of Nepal (pers. experiences).

At present, there are over a dozen commercial products based on nine principal fungal species registered worldwide for inundative use against invertebrate pests (Shah and Goettel, 1999). There is three primary application strategies utilised against insects such as, classical, inoculative and inundative approaches (Inglish *et al.* 2001). Entomopathogenic Hyphomycetes applied against insect pests using the inundative deployment strategy are frequently developed and assessed based on chemical pesticide model and in most instances, entomopathogens fit this model poorly. Inglish *et al.* (2001) mentioned this model as the application of a specific quantity of an active ingredient to an insect population with the goal of inciting catastrophic and rapid mortality, thereby quelling an outbreak. Entomopathogenic Hyphomycetes are often relatively slow –acting or their effects may be sublethal.

Under optimal conditions, the death of an insect usually takes between 3 and 5 days from the time of application. In field environments, the death can take substantially longer. This is usually due to conditions of suboptimal environment, which can prolong disease initiation and progress. Despite the delayed onset of mortality in field environments, the feeding behaviour of the insect pest can be affected during the period between infection and death. Although chemical pesticide models rarely take crop consumption into consideration in assessing efficacy, reduced feeding following the application of entomopathogenic Hyphomycetes can

have a significant impact on crop protection. Fragues *et al.* (1994) observed that, two days following application of *B. bassiana,* substantial reductions (57%) in consumption of foliage were observed in Colorado potato beetle larvae. Using faecal production as an indicator of food consumption, Thomas *et al.* (1997) also showed considerable reductions in feeding by grasshoppers (*Zonocerus variegates)* infected with *M. anisopliae* var. *acridum* prior to death (< 7days). A similar effect of *M. anisopliae* var. *acridum* on the acridid *Rhammatoverus schistoceroides* was observed in Brazil (Faria *et al.* 1999).

Insect pathogens can have a major role in pest management but their effective integration into such systems depends on their compatibility with the other components of the system. There have been numerous studies of the effects of agro-chemicals on entomopathogenic fungi (McCoy *et al.* 1988). Most studies have indicated that entomopathogenic fungi are compatible with many agrochemicals, including most insecticides (Benz, 1971) and with a number of beneficial insects (James and Lighthart, 1994). Fungicides are the most important exception (Jaros-Su *et al.* 1999). Only through careful field evaluation compatibility or incompatibility can be determined and field applications are already conducted in Switzerland (Keller *et al.* 1989).

### 2.1.17 Safety with entomopathogenic fungi

Until now there are over a dozen commercial products based on nine principal fungal species registered worldwide for inundative use against invertebrate pests (Shah and Goettel, 1999). No apparent detrimental effects have been reported due to their use. In considering safety toward all organisms, vertebrates, invertebrates and plants not intentionally being affected by the biological control fungus are referred to as “non target organisms” (Goettel *et al.* 1990). Cook *et al.* (1996), has mentioned the potential safety issues as proposed by the North Microbial Biocontrol Working Group. This includes competitive displacement of non target organisms, allergenicity, and toxigenicity to non-target organisms and pathogenicity to non-target organisms. They have reported that, *M. anisopliae* has been tested against several hosts, including cockroaches, grasshoppers, weevils and scarabs. This fungus has reported to be tested in mice and rats, rabbits and frogs (Burges, 1981; Shadduck *et al.* 1982; Saik *et al.* 1990) and birds (Smits *et al.* 1999). No adverse effects were reported and there was no evidence of infectivity. However, Mycotech observed extreme toxicity to mice by an isolate of *M. anisopliae* and one of *M. anisopliae* var. *acridium* (Goettel and Jaronski, 1997). Interestingly, Genthner and Middaugh (1995) reported that, in their laboratory assay system, *M. anisopliae* conidia adversely affected both embryos and newly hatched larvae of the inland silverside fish. Similarly, some cases of human infection have been reported. *M. anisopliae* has been registered and deemed safe when used according to the label of instruction (Goettel *et al.* 2001).

Safety of entomopathogenic fungi has extensively reviewed by Goettel *et al.* (1990), Prior (1990), Goettel and Johnson (1992). Guidelines for testing the pathogenicity and infectivity of entomopathogens to mammals have been reviewed by Siegel (1997) and guidelines for evaluating effects of entomopathogens on invertebrate non-target organisms have been reviewed by Hajek and Goettel (2000). Beneficial insects are generally not reported as being susceptible to *M. anisopliae.* It has been detected in nests of bumblebees but has not been reported causing disease in any bees including honeybees, *Apis mellifera* L. (Macfarlane, 1976), however, it is regarded as natural enemies of silkworm in Japan and India (Aoki, 1958;

Ganga and Chetty, 1999). No evidence has been found, *M. anisopliae* being pathogenic to birds, mice, rats, guinea pigs and rabbits (Austwick, 1980; Zimmermann, 1993) and earthworm (Hozzank *et al.* 2003).

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## Appendices

### Appendix 2.1.1 Most common hosts of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin

| Scientific name | Common name | References |
|---|---|---|
| *Adoryphorus couloni* (Burmeister) | Cockchafer | Rath *et al.* 1995 |
| *Amara obes* (Say) | Ground beetle | Rockwood, 1951 |
| *Adoryphus couloni* (L.) | Subterranean scarab | Rath *et al.* 1995 |
| *Amblyomma supinoi* (Neumann ) | Livestock mites | Kaaya *et al.* 1996 |
| *Anthonomus grandis* (L.) | Boll weevil | Camargo *et al.* 1985 |
| *Bemisia argentifolli* (Bellows and Perring) | Silver leaf white fly | Davidson *et al.* 1996 |
| *Brontispa longissima* (Gestro) | Coconut leaf beetle | Liu *et al.* 1989 |
| *Chalcodermus aeneus* (Horm) | Cowpea curculio | Bell and Hamelle, 1970 |
| *Carposina niponeniis* (Walshingham) | Peach fruit moth | Yaginuma and Takagi, 1987 |
| *Choristoneura fumiferana* | Spruce bud worm | Brousseau *et al.* 1996 |
| *Chilo saccharihagus* (Bojer) | Spotted borer | Sosa Gomez *et al.* 1996 |
| *Choristoneura fumiferana* (Cleman) | Spruce bud worm | Brousseau *et al.* 1996 |
| *Coptotermes formosanus* (Shiraki) | Sub terrain termites | Lai *et al.* 1982 |
| *Curculio caryae* (Horn) | Pecan weevil | Latch and Fallon, 1976 |
| *Curculio caryae* (Horn) | Weevil | Saito and Suzuki, 1982 |
| *C. camelliae* (Roelofs) | Goat moth | Madelin, 1963 |
| *Dendroctonus frontalis* (Zimmermann) | Southern pine beetle | Davidson *et al.* 1996 |
| *Frankliniella occidentalis* (Pergande) | Western flower thrips | Helyer *et al.* 1995 |
| *Glossina pallidipes* (Austen) | Tsetse fly | Kaaya and Munyinyi, 1995 |
| *Gonocephalum macleayi* (Blackburn) | False wireworm | Robertson, 1993 |
| *Hyphenemus hampei* (Ferari) | Coffee berry borer | Rosa *et al.* 1997 |
| *Hylobias pales* (L.) | Pales weevil | Madelin, 1963 |
| *Ixodes scapularis* (Say) | Questing adult | Benjamin *et al.* 2002 |
| *Laodelphax striatellus* (Fallen) | Smaller plant hopper | Liu *et al.* 1989 |
| *Lygyrus subtropicus* (Blatchley) | Sugarcane grub | Raid and Cherry, 1992 |
| *Marhanarva posticata* (F.) | Sugarcane spittle bugs | Roberts and Yendol, 1971 |
| *Melanoplus sanguinipes* (F.) | Grasshopper | Bidochka and Khachatourians, 1994 |
| *Melolontha melolontha* (L.) | European chafer | Keller, 2000 |
| *Musca domestica* (L.) | Housefly | Barson *et al.* 1994 |
| *Nasutiterenes exitiosus* (L.) | Termite | Aizawa, 1987 |
| *Nilaparvata lugens* (Stal) | Brown plant hopper | Thi *et al.* 1994 |
| *Nomadacris septemfasciata* (Serv) | Red locust | Arthurs and Thomas, 2001 |
| *Oryctes rhinoceros* (L.) | Rhinoceros beetle | Latch and Fallon, 1976 |
| *Otiorhynchus salcatus* (F.) | Vine weevil | Moorhouse *et al.* 1993 |
| *Palaemonetes pugio* (Holthius) | Grass shrimp | Genthner *et al.* 1997 |
| *Phyllophaga smithi* (Arrow) | White grubs | Rath *et al.* 1995 |
| *Rhipicephalus appendiculatus* (Newmann) | African mite | Kaaya *et al.* 1996 |
| *Spodoptera exigua* (Hubner) | Beet army worm | Raid and Cherry, 1992 |
| *Tomaspis saccharina* (Distant) | Sugarcane frog hopper | Madelin, 1963 |
| *Austrinia nubilalis* (Hubner) | European corn borer | Madelin, 1963 |

## SECTION 2.2 THE WHITE GRUBS

### 2.2.1 Introduction

The world fauna of Scarabaeidae exceeds 30,000 species (Mittal, 2000) with about 1300 North American species (Borror *et al.* 1975). The maximum number occurs in the tropical areas of the world, particularly in African and Oriental regions. The fauna of Indian sub region is very rich and diverse, but it is yet to be fully explored (Richter, 1971; Mishra and Singh, 1999). The larvae of lamellicorn beetles are called white grubs. Various types of grubs in terms of morphology, occurrences, species etc. are observed in different agro-environment. Among those commonly available are the masked chafers, *Cyclocephala* spp (annual grubs); May/June beetles, *Phyllophaga* spp (three-year grubs) and most recently the Japanese beetle, *Popillia japonica*. True white grubs are the larvae of May beetles (also called June Beetles) found in the genus *Phyllophaga*, of which there are over 100 different species. White grubs in the genus *Phyllophaga* are also known as "perennial" white grubs or "true" white grubs. *Phyllophaga* larvae and other larvae of the family Scarabaeidae are often referred to as "white grubs", including larvae of the Japanese beetle (*P. japonica)*, annual white grubs (*Cyclocephala* spp.), and the green June beetle (*Cotinis nitida*). Some of them could be facultative pests and other saprophytic (mainly at sub-family level) however, the species that cause damage (or “pest” species”) in Nepalese farming are largely unknown.

### 2.2.2 Systematic position

Table 2.2.1 Systematic position of white grubs as proposed by Arrow (1917).

| | |
|---|---|
| Order | Coleoptera |
| Sub-order | Polyphaga |
| Series | Lamellicornia |
| Family | Scarabaeidae |

The family Scarabaeidae is divided into the following important sub-families such as:

1) Scarabaeinae: (= Coprinae): Dung beetles (Genus: *Copris, Catharsius, Onitis, Phalops Caccobius, Onthophagus, Drepanocerous, Oniticellus, Liatongus, Serica* etc) and Tumble beetles.
2) Cetoniinae: Flower beetles, sap chafer (Genus: *Jumnos, Protaetia, Cinteria* etc.)
3) Dynastinae: Rhinoceros beetle, Hercules, Elephant beetles (Genus: *Xylotrupes, Oryctes, Phyllognathus* etc.)
4) Rutelinae: Shining leaf chafers (Genus*: Anomala, Adoretus, Popillia, Mimela)*
5) Melolonthinae: June beetles, May beetles, cockchafers (Genus: *Melolontha, Brahmina, Articephala, Holotrichia, Hilyotrogus, Cryptotrogus)*
6) Troginae: A gnawer (*Trox indicus*)
7) Geotrupinae: *Odontaeus, Geotrupes*
8) Hyposorinae: *Hyposorus*
9) Aphodiinae: Small dung beetles, *Aphodius*

Most common pest species of white grubs are presented in detail in the Appendix 2.2.1

### 2.2.3 Morphology

The members of the Scarabaeidae family vary greatly in size (about 0.5 mm up to 150 mm body length in the longest) and they show a great diversity in shape, coloration, and sculpture (Crowson, 1981 and Hlavac, 1973). The scarabs are heavy bodied, oval or elongated usually more or less convex, varying enormously in size and chiefly distinguished by having the lamellae at the tips of the antennae. Their organs of smell are located on the movable lamellae or leaves on the thickened end of the antennae (Linsenmair, 1972). The last antennal segments are expanded into plate-like structures that may spread apart or united to form a compact terminal club, movable and capable of being brought close together or separated. The anterior coxal cavities are large, transverse, closed behind the pygidium is usually exposed, abdomen as the rule with six or seven visible ventral segments. The legs are fossorial but variable, tarsi five segmented, the anterior pair sometimes absents.

Colours are of all imaginable hues and combination from dull and somber to the most brilliant or jewel-like with metallic and enameled surface. The typical species rival all other objects in nature for beauty of colour and design (Essig, 1982). Larvae have a well-developed yellow to reddish-brown sclerotized head, C-shaped bodies, usually three pairs of thoracic legs, usually a 10-segmented abdomen, and spiracles on the mesothorax, rarely on prothorax, and first eight abdominal segments (Booth *et al.* 1990). The pupae are free and usually occur in the normal habitat of the larvae, some are enclosed in a cell composed of surrounding materials. The species have predominant dimorphic forms but in the genera *Anomala* and *Adoretus* there is difficulty to distinguish as the males and females resemble each other. The other groups can be easily differentiated by the obvious sexual features like the horns or mandibles (Arrow, 1917). The claws and feet of the males are remarkable which facilitate the grasping (Flower, 1917). White grubs are identified by different ways; however, the arrangement of bristles and hairs on the underside of the tip of the abdomen, called the raster pattern is also taken as an important key for identification.

### 2.2.4 General life cycle

The biology of the insect has been reported by Veeresh (1977), Patil and Hasabe (1981) and Raodev *et al.* 1974). Different species of white grubs have similar patterns of life cycle but may vary according to the climatic factors at the time of emergence, egg lying, active larval period, time of pupation and other stages (Sharma, 1989). Matheson (1985), reported the life history of some of the beetles which take more than three years in temperate regions and two years seems to be the normal. Some appears every year, though indicating the existence of three broods in those regions where three- year life cycle exists (Brink, 1955) but the size of the broods may be markedly different and the injury varies correspondingly. Every third year, the most abundant brood will cause the heaviest damage.

Majority of the beetles are closely related in respect of their taxonomic characters, however, they are distinct biologically. Few species may complete their life cycle in one year e.g. all the known species of *Holotrichia* (Yadav and Mathur, 1987) the others like

European cock chafer *Melolontaha melolontha* has at least of three years cycle (Keller, 2000) and many others have biannual cycle.

2.2.4.1 Adult emergence

Normally in India, adult beetles emerge from the soil during April-June in response to the first seasonal rains (Yadava and Mathur, 1987; Singh and Mishra, 2003). Emergence takes place at dusk between 17.45 and 18.45 h at 27-30°C. There are some reports that it needs some rain at least 11 mm on the last week of May (Mishra and Singh, 1999). The second fortnight of June observed is the peak period of emergence of the June beetles and emergence continued until the fortnight of August (Mittal, 2000). Rainfall, temperature, atmospheric humidity and wind velocity largely govern the emergence, movement and distribution of adults (Mishra and Singh, 1999). It has also been observed that the particular intensity of light at dusk also triggers the emergence of adults. The adult beetles strip the foliage of the shade trees, crop plants or any forest trees.

2.2.4.2 Oviposition

Females crawl or fly to a low branch or other support, where they hang with the tip of the abdomen extruded. Males emerge shortly afterwards and mate for 10-15 minutes after a short searching flight, hanging inverted from the female genitalia. The adults mate in the evening and at dawn. Females return to the ground to deposit the eggs in the soil depending upon the softness of the soil. Repeated mating and continued feeding are necessary to ensure both a high fecundity and fertility. The oviposition period ranges from about 50-100 days; fecundity varies from 0-140 per female. Since the adults are attracted to trees to feed, they tend to lay most eggs in the higher portions of sod near wooded areas (Potter, 1998).

The female beetles of *Holotrichia consanguinea* Blanch. and *Leucopholis lepidophora* Blanch. deposit their eggs singly in the ground near suitable roots. The total number of eggs by one individual being nine to thirty per laying time and they lay white eggs at 1-8 inches depth inside the soil (Yadava and Vijayavergia, 1994). Whereas, the adult females of *Melolontha melolontha* (L.) lay eggs in groups of 12-46 (Keller, 2000). The eggs increase considerably in size before hatching, growing in a few days to more than double their original weight and bulk through the absorption of moisture by the albumin. The elongated-oval eggs become exactly spherical after the absorption of the water. The larvae hatch within two weeks during June and the growth of the larvae start feeding upon the roots. The eggs are oval creamy white when freshly laid and later turn to brown in colour. The larvae hatch 6-13 days later. The adult June beetles prefer to oviposit in loose grassy sod or weedy land. *Canthon* spp lays the eggs on the dung ball and developing larvae utilize it as food (Ross, 1956).

2.2.4.3 Larvae

After hatching the larvae, immediately burrow into the soil and begin feeding on organic matter and small roots. The newly emerged grubs are of different color, the grub species of *Holotrichia* are creamy white. There are three instars of the pest and the mean body length and width varies with the species (Yadava and Mathur, 1987). The larval duration for the first and second instar is short as compared to the third instar (Singh and Mishra, 2003).

Larvae are C-shaped and extremely vulnerable at this stage and even slightly unfavorable environmental conditions may cause up to 75% mortality. The legs are well developed and often hairy. The head is large, hypognathous and heavily sclerotized, yellow-brown or red-brown in color, and with powerful, exposed mandibles. All larval stages live in the soil and are phytophagous, feeding upon living plant roots, ingesting at the same time quantities of soil and organic matter. They migrate downward to a depth of up to 1.5 meters and remain inactive until the following spring. At this point in the insect's life cycle the greatest amount of damage occurs as the larvae return near the soil surface to feed on the roots of the plants. The next autumn the larvae again migrate deep into the soil to over- winter, returning near the soil surface the following spring (the third spring) to feed on plant roots until they are fully grown in late spring. Third-instar larvae, which cause more damage to host plants than those in earlier instars, are present from the end of June or July until October. Full-grown larvae burrow deeper into the soil and form a cell in the first compact soil they encounter, usually at a depth of 20-30 cm. This takes place from August onwards and is completed by all individuals by November. These grubs then form oval earthen cells and pupation take place in February or March under field conditions, but may occur earlier in the laboratory.

2.2.4.4 Pupation

The fully grown larvae burrow deeper in the soil and construct an earthen cell in which it passes a quiescent or prepupal stage which last for one to six weeks. The pupation is characteristics for each species (Nayar *et al.* 1976). This stage lasts 30-40 days at 23-25°C soil temperature and about 30 cm below the surface of an undisturbed soil. The freshly formed pupa is light yellow and extremely tender, but as it grows older it turns brown and hardens. It is of exarate type and mortality may be as high as 25% in disturbed soil conditions. The pupal stage lasts from 10- 27 days depending upon the type of soil, the species involved and the agro-ecological conditions. The majority of the root grubs pupates during November- December, however it may also take place during September and February (Yadav and Mathur, 1987). The adult matures and remains quiescent until the cell is broken artificially or emergence is induced by rainfall soaking the soil. Emergence may therefore be synchronized by the first rains in May or June when feeding, mating, and egg-laying take place. However, *P. elenans* may take two years to complete the life cycle (King, 1984).

### 2.2.5 Damage symptom

The adults of the green beetle (*Anomala dimidiata* Hope) feed the whole leaves and flowers (field observation, 2005) whereas, adults of European chafer, *Melolontha melolontha* (L.) feed from the margins without leaving the midribs or stout veins causing host plants completely denuded (Keller, 2000). The larvae of the Chinese rose beetle, *Adoretus sinicus* Burmeister feed on the primary roots, rootless, root hairs, tubers and rhizomes of variety of plants. In the tuber crops like potato, the grubs make large, shallow and circular holes and render them unfit for consumption (Singh and Mishra, 2003).

The infested plants show stunted growth, wilting appearance and finally dry up (Metcalf and Flint, 1975). Such plants can be easily pulled out from the soil. The severely infested fields show patchy appearance due to withering or drying up of the plants. A good indication of grub infestation is the presence of skunks, crows, mynas and moles feeding on

fields following the plough. Symptoms are similar to *Phytophthora* root rot, drought damage, and improper planting, specially the tree sets of one or two years. To distinguish between these problems, pulling up the affected plants and examining roots, looking for the loss of roots due to grub feeding and digging in the soil around these trees to look for grubs is recommended. The damage to crops may be as high as hundred percent in certain areas (Yadav and Mathur, 1987).

### 2.2.6 Host plants

The larvae prefer to feed upon the plant roots of corn, groundnut, potatoes, strawberries and several other hosts, however, they dislike legumes (Matherson, 1985), the sweet clover (Metcalf and Flint, 1975). The larvae of *M. melolontha* do their greatest injury in their second year but in the third year may sometimes damage early plantings (Keller, 2000). The grubs also feed on the roots of crops like upland rice, finger millet, foxtail millet, French bean, soybean, amaranthus etc. The groundnut and chilies are more susceptible as compared to pearl millet, sugarcane and sorghum. In general tap root system are more susceptible as compared to adventitious roots. The non-structural carbohydrates found within the plant stimulates feeding in the beetle (Arita *et al.* 1993).

The grubs are subterranean and attack a wide range wild plants and crops such as potato, chilies, tomato, okra, brinjal, ginger, and Cole crops are the major vegetables severely damaged (Singh and Mishra, 2003). Almost all field crops growing during the rainy season are damaged. According to Bhatacharjee and Bhatia (1981); Rohilla *et al.* (1981); Crocker *et al.* (1990); Baksha (1990) and Guo and Wen (1988), primary hosts are *Arachis hypogaea* (groundnut), *Sorghum bicolor* (sorghum), *Zea mays* (maize), *Saccharum officinarum* (sugarcane), *Glycine max* (soyabean), *Vigna mungo* (black gram), *Vigna radiata* (mung bean), *Cajanus cajan* (pigeon pea), cucurbits, Brassica, *Solanum melongena* (aubergine), *Lycopersicon esculentum* (tomato), *Ipomoea batatas* (sweet potato), *Cyamopsis tetragonoloba* (clusterbean), *Vigna unguiculata* (cowpea), *Helianthus annuus* (sunflower), *Jasminum* (jasmine), *Nerium*, *Pinus resinosa* (red pine), *Larix leptolepis* (Japanese larch), *Acacia decurrens* (green wattle), *Solanum tuberosum* (potato), *Zingiber officinale* (ginger), *Pennisetum glaucum* (pearl millet), *Capsicum annuum* (bell pepper), *Brassica rapa (*turnip), *Raphanus sativus* (radish), *Abelmoschus esculentus* (okra), *Tectona grandis* (Indian oak), *Avena sativa* (oats), *Triticum* (wheat), *Hordeum vulgare* (barley), *Albizia*, *Leucaena leucocephala* (Leucaena), *Swietenia* (mahogany), *Coffea* (coffee).

Similarly, secondary hosts are *Panicum maximum* (Guinea grass), *Ricinus communis* (castor bean), *Sesamum indicum* (sesame), *Crotalaria juncea* (sun hemp), *Gossypium* (cotton), *Prunus dulcis* (bitter almond), *Vitis vinifera* (grapevine), *Grewia asiatica* (phalsa), *Ficus carica* (fig), Prunus (stone fruit), *Mangifera indica* (mango), *Citrus* sp (citrus), *Eleusine coracana* (finger millet), turf grasses. In addition there are some wild hosts such as *Azadirachta indica* (neem), *Acacia nilotica* (scented thorn), *Ficus spp, Lagerstroemia indica, Eucalyptus citriodora* (lemon-scented gum), *Ziziphus jujuba* (common jujube), *Psidium guajava* (guava), *Morus alba* (mora), *Acacia* (wattles). The grubs feed on roots of almost all the crops like jowar (*Sorghum bicolour*), bajra (*Pennisetum typhoides* L.), sesame (*Sesamum indicum*), potato (*Solanum tuberosum)*, sunflower (*Helianthus annuus*), chilies (*Capsicum frutescens*), cotton (*Gossypium* spp.), sugarcane (*Sachharum officinarum*), tobacco (*Nicotiana tabacum*), brinjal (*Solanum melongena*), cucurbit

(*Cucumis sativus*), lady's finger (*Abelmoschus esculentus*), sugar beet (*Beta vulgaris*), barley (*Hordeum vulgare*) etc.

Beetles are nocturnal in habit and defoliate the foliage of the plants during night hours. They attack at any stages such as vegetative or growing or flowering stage and fruiting stage on leaves, roots, inflorescence, and fruits/pods. The adults also feed on wild rose, yellow raspberry, black raspberry, wild grapes and the ornamental plants like rose dahlia, gladiolus and field crops like tassels of maize, French bean, soybean etc (Habeck, 1963). The beetles sometimes gnaw the floral parts and immature fruits of apple, peaches, pear, walnut etc (Fleming, 1972). The adults of *Anomala lineatopennis* feed on the ripe fruits of peach in the June- July (Singh and Mishra, 2003). The adults of Chinese beetle, *Adoretus sinicus,* feed on foliage and fruits of about two hundred fifty five plant species including nearly all the deciduous and small fruits, shade trees, shrubs, corn, soybean, garden flowers, vegetables and weeds. Similarly, two hundred seventy seven plant species are affected by *Popillae japonica* (Hawley and Metzger, 1940; Fleming, 1972). Detailed of the host plants of the larvae is presented in Appendix 2.2.1.

### 2.2.7 Influence of soil factors

The light soil provides the optimal condition for the survival and multiplication of the many pests (Blossey and Joshi, 2003). Wherever, in Nepal such soils have brought under cultivation with better irrigation facilities, the grubs gradually multiplied and become the serious threat to crops in the areas (pers. experiences), a similar situation is also reported in India by Singh and Mishra (2003). The heavy and the waterlogged soil are not suitable for the survival of the white grubs (Cherry, 1984b).

On the other hand, the easy availability of plants for feeding to the adult in the infested areas contributes to the increased severity of the pest (Terry *et al.* 1993). The pest problem remains concentrated in the vicinity of the host trees. Thus the assured supply of food for the larvae and for the adults and the well drained light soil is a prerequisite to get pest status (Blossey and Joshi, 2003). The change of cropping pattern to grow fodder, forage and the cereals crops round the year provide ideal condition for the survival and the multiplication of the pest (Keller *et al.* 2000). Weather that puts trees under stress, such as hot, dry weather, will increase aboveground symptom expression of grub damage since the root system is not able to support the tree (Blossey and Joshi, 2003). Droughts can also kill grass ground covers leaving nothing else for grubs to eat except tree roots.

### 2.2.8 White grub management

Since 1888, entomologists all over the world have made attempts to control white grubs by adopting mechanical, biological, cultural and chemical control methods since no single control measure is effective. Early methods of scarab control relied on non-chemical and cultural practices. Adults of large species were hand collected with varying reports of success (Dumbleton, 1942; Zimmermann, 1992). Trapping has also been widely used in the past. Langford *et al.* (1940) reported on the capture of 100 tonnes of Japanese beetles in Maryland in 1939 with the use of geraniol traps. Cultivation and stock management have also been used for scarab control (Jackson *et al.* 1989). In the absence of effective control, farm management practices have been altered to reduce the risk of scarab attack (Flay and Garrett, 1942).

Damage may be compensated by the addition of nitrogenous fertilisers (Prestidge and East, 1984). In countries with an abundance of labour, such as India, a wide range of cultural techniques, including mechanical collection of adults and cultivation methods to kill larvae, provide the basis for scarab control (Anon., 1985). Similar practice could be adopted in Nepal, where agricultural labour forces are cheaply available.

The pest can be managed only with integration of various control measures (Kaunsale *et al*. 1978; Keller, 2000, Singh and Mishra, 2003). A prerequisite is the knowledge of the economic threshold. This parameter is dependent mainly on species, larval instar, crop and environmental conditions. That's why there in no unique threshold. Flemming (1972) reported that densities of 11 or more grubs of *P. japonica* $m^{-2}$ cause significant damage to drought stress turf. On the other hand, Schweigkofler and Zelger (2002) mentioned that summer density of 1-4 grubs $m^{-2}$ cause little damage to agriculture, whereas, 20-30 $m^{-2}$ caused considerable economic loss. In Nepal white grubs management based on economic threshold level has not been started yet and probably it may be different in respect to time and place, and species involvement.

2.2.8.1 Mechanical control

Various workers have reported that hand collection of beetles after emergence is one of the cheapest, simplest and best methods to minimize the white grub population. Beetles that have congregated on host trees are collected by hand or the host trees are jerked with the help of a hooked bamboo pole. The beetles that fall on the ground are picked up in the petromax light and put into a kerosene-water mixture to be killed. Mechanical collection should be done for 4-7 days, depending on the beetle population after each day's emergence. Gupta (1973) captured 70,000-85,000 adults of *Holotricha serrata* by planting 1.25-2.00 m long fresh branches of *Azadirachta indica* in the infested areas at the rate of two per hectare daily during the months of June and July.

Light traps can be used for collecting adult beetles during the night. At the time of emergence, spraying of host trees with DDT, carbaryl, fenitrothion, monocrotophos, endosulfan or quinalphos markedly reduces the beetle population (Yadava *et al.* 1987). The use of nets to protect expensive crops is also an effective non-chemical control measure (Schmid, 2000; Brenner and Keller, 1996). The use of nets strongly decreases the density of cockchafer larvae population during following years. The flower and fruit cluster can be bagged with cheesecloth to protect them from damage of adults. A perforated, sticky plastic sheet placed under the canopy of a tree captured more beetles than 15-W black light survey trap. A sticky sheet is an alternative, less expensive, equally effective survey tool for detection of European chafer infestations (Fiori, 1983). However, their use in commercial scale is not feasible in the long term and also causes environmental hazards. Therefore, the biocontrol agents especially the pathogens could be one of the best alternatives for their sustainable management (David *et al.* 1967).

2.2.8.2 Cultural control

Cultural techniques are useful in reducing the number of larvae as well as of adult populations. Different cultural practices, such as ploughing, harrowing, hoeing, flooding and fallowing of fields, trap cropping and crop rotation, have been suggested by various

workers. Yadava *et al.* (1987) suggest different tillage operations for the control of the various stages of the pest. David and Kalra (1966) suggested flooding for a long period to obtain some relief from white grub. They advised crop rotation with sugarcane-paddy-sugarcane in endemic areas as a mean of control. Avasthy (1967) reported that fallowing of land for two consecutive years helped in reducing the pest. Growing of resistant crops such as sunflower also checks the build-up of grub populations (Veeresh, 1974). He also suggested sowing of trap crops such as sorghum, maize, onion etc. to reduce white grub infestation. Labana (1987) reported that the soil should be ploughed twice during May and June at or before sowing to expose white grubs in the soil to harsh weather. In endemic areas summer ploughing exposes the larvae of different stages which may then eaten by birds. Deep ploughing in August-September to expose the third instar grubs for predatory birds is quite effective in reducing the grub population (Singh and Mishra, 2003). They have further reported that the use of nitrogenous fertilizers, especially ammonia and urea, at high dosages kill the first-instar larvae, however, the partly decomposed farmyard manure provides congenial condition of surviving newly emerged grubs of saprophytic species. Necessity on the well decomposed farmyard manure (G. C. and Keller, 2002) is widely recommended in Nepal looking into the incorporation of farm yard manure (FYM) into the farmlands during maize sowing.

2.2.8.3 Chemical control

White grubs are subterranean and hence difficult to control. However, various workers have used different chemical substances for their control. David and Kalra (1966) found that soil application of insecticides such as phorate, quinalphos or gamma-HCH dust before sowing summer crops gave satisfactory control of the pest. Veeresh (1974) reported that phorate gave highly significant control of grubs. Bhatnagar (1975) used gamma-HCH to control the pest in groundnut crops. Veeresh *et al.* (1977) found carbofuran and phorate effective in controlling the grubs. Patil *et al.* (1991) observed that soil application of carbofuran or phorate or soil drenching with chlorpyriphos as effective for control of the pest. Veeresh (1974) suggested drenching with chlorpyriphos for economic and effective control of the pest. As an alternative to mechanical collection of beetles, the spraying of host trees such as *Acacia nilotica* and *Azadirachta indica* has also been suggested. Bindra and Singh (1971) reported spraying of carbaryl and fenitrothion for the control of beetles. Raodev *et al.* (1976) observed that one spraying of 12,000 *Acacia nilotica* and *Azadirachta indica* trees with DDT killed 88% of beetles and led to a reduction of 80% of the larvae of *H. serrata.*

In Nepal, the use of insecticides is the readily available and dominant means of pest control because of the lack of alternative measures. This situation is mainly due to poorly developed infrastructure and lack of trained manpower in the country. As a result the farmer is compelled to use highly persistent and ecologically destructive pesticides. In some instances banned insecticides such as BHC (class II, moderately hazardous, banned in 28 countries) followed by metacid (class 1a, extremely hazardous, banned in five countries) (Dahal, 1995) are still been used. Farmers have popular misconception that pesticides are the “medicines” to kill pests not as “poison” that affects other living organisms. Because of this, farmer first attempt to use chemicals rather than seeking other alternatives. Hazards due to pesticides related problems are increasing at many levels and it is very common while attempting soil insect pests. These factors have lead the researchers to focus on the development of alternative control measures.

2.2.8.4 Biological control measures

A number of natural enemies such as the insect parasitic nematodes, fungus to control white grubs and bacteria are reported to be used with partial or moderate successes in different parts of the world (Appendix 2.2.2). Numerous species of mammals, birds and insect are also emphasised, however, it is difficult to exploit them as other bio agents.

*Nematodes*

Nematodes are widely used to control garden chafers *Phyllopertha horticola, M. melolontha* and *Amphimallon solstitiale* on meadows and gulf courses (Peters and Galarza, 2002). The nematode *Heterorhabditis bacteriophora* was mass reared in liquid culture and commonly used as "nema-green". *Steinernema glaseri* cause significant mortality of second instars larvae of *M. melolontha* at 1500 nematodes per grubs (Peters, 2000). The use of entomogenous nematodes *Neoaplectana carpocapsae* and *H. heliothidis* at 12.35 x $10^{14}$ nematodes per ha with irrigation following application and moderate soil moisture produced 53 and 73 % control of Japanese beetle, *P. japonica,* larvae in golf course turf (Shetlar *et al.* 1988).

The dispersal of *H. bacteriophora* in the soil depends on the presence, density or distribution of Japanese beetle grubs and in turn activity and behaviour of the grub is affected by the presence of nematodes (Schroeder *et al.* 1993).

*Bacteria*

The bacteria *Serratia entomophila* (Jackson *et al.* 1992) and *Bacillus popillae* are reported to cause the lethal disease of scarab larvae which is characterized by a mass development of sporangia in the haemolymph. Bacteria infect insects through the mouth and digestive tract. *B. popillae* produces a crystal protein during sporulation (Dutky, 1963). The bacteria grow and sporulate within living host over a relatively long period. These products are successful and widely available as liquid concentrated, wettable powders, and ready- to use dusts and granules (Julian and Hall, 1968).

*Fungus*

The insect pathogenic fungi *M. anisopliae* and *B. brongniartii* (Keller, 2000) have been reported throughout the world. Fungus based natural enemies have successfully been applied in countries like Switzerland, Austria, New Zealand and Australia (Keller, 2000; Strasser, 1999; Jackson *et al.* 1992; Rath *et al.* 1995). After application, the fungi persisted in the soil due to their capacity to multiply in the host (Fox, 1949). They are also easily isolated from the soils (Zimmermann, 1993) and insects (Madelin, 1963). *Cordiceps aphidii* is reported from the larvae of the pasture cockchafer *Aphodius howittii* (Steinhaus, 1949).

*Viruses*

Viruses from several families cause disease in species of Scarabaeidae (Glare and Crawford, 1992) however, little attention has been given. There are nine virus families with members known to replicate in insects. There are Baculoviridae, Reoviridae, Poxviridae,

Iridoviridae, Picornaviridae, Rhabdoviridae, Polydnaviridae (Payne and Kelly, 1981) and Nodaviridae (Scherer and Hurlbut, 1967, Matthews, 1982).

More than 1600 virus have been recorded in insects (Martignoni and Iwai, 1986). The majority of insect viral diseases have been described from Lepidoptera, relatively few virus have been isolated from species of Scarabaeidae. The detailed of the beetle species that are infected with several viruses is presented in Appendix 2.2.3.

2.2.8.5 Integrated management

The scenario of soil pest management in most parts of the developed agriculture has been changed from single control to integrated approach (Bednarek, 2000) especially after the development of resistance, resurgence and several levels of environmental mis-haps. Combined approaches including microbial pesticides, cultural methods, mechanical and physical are well accepted methods. For the reduction of the white grub problems scientists from several parts of the world have emphasized several measures. Use of well decomposed farm yard manure (G. C. and Keller, 2002), crop rotation and mixed cropping with sweet clover, horse gram and others like *Amaranthus* and sweet potato (Matheson, 1985) and clean cultivation (Yadava and Vijayavergia, 1994) were reported to be useful for the reduction of pest density. Flooding (Cherry, 1984a) and balanced use of fertilizers (Brady, 1996) can also be adopted for white grub reduction. Use of malt extracts in combination with light traps is less useful mechanical means for the reduction of *A. dimidiata* and *H. longipennis* (Fiori, 1983). Use of fungal pathogens (Keller, 2000; Schweigkofler and Zelger, 2002) with different formulations such as fungus colonized grain or spore suspension (Keller *et al.* 1997). New methods like application of spore powder during ploughing are still to develop. Niemczyk and Lawrence (1973) reported resistance developed by white grubs to several groups of insecticides which therefore may be applied only as a last alternative in combination with other compatible methods. The combined treatment with fungi *B. brongniartii* (Sacc.) Petch or *M. anisopliae* (Metsch.) Sorokin or nematodes (*Heterorhabditis megidis* Poiner), and the insecticide carbofuran increased the mortality of *M. melolontha* (L.) larvae more than any of these agents alone (Bednarek *et al.* 2000).

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## Appendices

### Appendix 2.2.1 Most common pest species of white grubs (Scarabaeidae) to different host plants

| Scientific name | Host plants | References |
|---|---|---|
| *Anomala antiqua* (Gyll.) | Paddy, maize, cassava | Kalshoven, 1981 |
| *Anomala aureola* (Hope). | Golf grasses, ferns | Kalshoven, 1981 |
| *Anomala dimidiata* (Burm.) | Polyphagous | Shah and Garg, 1985 |
| *Anomala innuda* (F.) | Polyphagous | Beard, 1956 |
| *Anomala lucicola* (F.) | Polyphagous | Beard, 1956 |
| *Anomala oblivia* (Horn) | Polyphagous | Beard, 1956 |
| *Anomala obsoleta* (Blanch.) | Cassava | Kalshoven, 1981 |
| *Anomala orientalis* (Waterhouse) | Sweet potato | Oya, 1996 |
| *Anomala pallida* (F.) | Paddy, maize, cassava | Kalshoven, 1981 |
| *Anomala rugosa* (L.) | Polyphagous | Oya, 1996 |
| *Anomala simicus* (Blanch.) | Polyphagous | Cherry, 1984 a |
| *Anomala similis (*Mos.) | Adults on tree leaves | Cherry, 1984b |
| *Anomala cuprea* (Blanch.) | Sweet potato | Oya, 1996 |
| *Anomala rufocuprea* (Blanch.) | Soyabeans | Oya, 1996 |
| *Anomala varicolor* (Gyll.) | Sugarcane, maize, oats | Kalshoven, 1981 |
| *Anomala anchoralis* (Lab.) | Cocoa, cassava, lawns | Kalshoven, 1981 |
| *Anomala viridis* (F.) | Paddy, maize, sugarcane | Kalshoven, 1981 |
| *Adoretus sandaicus* (Ohs.) | Grape vine and Falsa | Kalshoven, 1981 |
| *Adoriphorus couloni* (L.) | Turfgrass | Yadava and Mathur, 1987 |
| *Amphimallon majalis* (Razoum.) | Polyphagous | Tashiro and White, 1954 |
| *Aphodius hoeitti (Hope)* | Polyphagous | Beard, 1956 |
| *Apogonia destructor* (Bos.) | Sugarcane and other crops | Kalshoven, 1981 |
| *Apogonia similis* (Mos.) | Adults on tree leaves | Kalshoven, 1981 |
| *Brahmina pubiventris* (Burm.) | Cocao, Kapok | Kalshoven, 1981 |
| *Cyclocephala immaculata* (Oliv.) | Turfgrass | Downing, 1994 |
| *Chaetadoretus bornensis* (F.) | Polyphagous | Kalshoven, 1981 |
| *Chaetadoretus sciurinus* (Burm.) | Polyphagous | Kalshoven, 1981 |
| *Costelytra zealandica* (White) | Pasture grass | Prestidge *et al.* 1984 |
| *Cyclocephala borealis* (Arrow) | Turfgrass | Downing, 1994 |
| *Cyclocephala parallela* (Casey) | Sugarcane | Cherry, 1984a |
| *Dermolepidia pica* (Arrow) | Cotton, coconut, sugarcane | Kalshoven, 1981 |
| *Exopholis hypholeuca* (Wied.) | Ground nut, tea, cassava | Kalshoven, 1981 |
| *Exopholis costata* (Burm.) | Rubber | Kalshoven, 1981 |
| *Heteronychus sanctae-helenae* (Bl.) | Polyphagous | Beard, 1956 |
| *Holotrichia coriacea* (Hope) | Potato | Mishra and Singh, 1999 |
| *Holotrichia longipennis* (Blanch.) | Polyphagous | Yadava and Mathur, 1987 |
| *Holotrichia setticollis* (Moser) | Summer and winter crops | Shah and Garg, 1985 |
| *Holotrochia consanguina* (Blanch) | Sugarcane | Gupta and Avasthy, 1956 |
| *Hylamorpha elegans* (Burmeister) | Polyphagous | Beard, 1956 |
| *Lepidiota stigma* (F.) | Sugarcane, maize, coffee | Kalshoven, 1981 |
| *Leucopholis cretacea* (Burm.) | Upland rice, rubber | Kalshoven, 1981 |
| *Leucopholis emarginata* (Burm.) | Pepper plantation | Kalshoven, 1981 |
| *Leucopholis lepidophora* (Banch.) | Polyphagous | Yadava and Vijayavergia, 1994 |

| | | |
|---|---|---|
| *Leucopholis rorida* (F.) | Cassava, sweet potato | Kalshoven, 1981 |
| *Leucopholis tristis* (Brsk.) | Polyphagous | Kalshoven, 1981 |
| *Lichnanthe vulpina* (Hentz) | Polyphagous | Essig, 1982 |
| *Ligyrus subtropicus* (Blatchley) | Sugarcane | Cherry, 1984a |
| *Maladera castanea* (Blanch.) | Sweet potato | Oya, 1996 |
| *Maladera matrida* (Argaman) | Sweet paotato | Golberze *et al.* 1989 |
| *Melolontha melolontha* (Linnaeus) | Meadows and turfgrass | Keller, 2000 |
| *Microtriehia sharpi* (Brsk.) | Gambir plantation | Kalshoven, 1981 |
| *Mimela fulgidivittata* (Blanch.) | Summer and winter crops | Shah and Garg, 1985 |
| *Odontria zealandica* (White) | Turfgrass | Beard, 1956 |
| *Oryctes rhinoceros* (Linnaeus) | Coconut | Beard, 1956 |
| *Oryctes nasicornis* (Linnaeus) | Coconut | Kalshoven, 1981 |
| *Phyllognathus dionysius* (F.) | Upland rice, maize | Kalshoven, 1981 |
| *Phyllophaga severini* (Brsk.) | Coffee plantations | Kalshoven, 1981 |
| *Phyllophaga koehleriana* (Saylor) | Range land grass | Daniels, 1966 |
| *Phyllophaga bipartita* (Horn) | Polyphagous | Guppy, 1982 |
| *Phyllophaga congrua* (LiConte) | Polyphagous | Guppy, 1982 |
| *Phyllophaga crassissima* (Blanch.) | Polyphagous | Hamilton *et al.* 1971 |
| *Phyllophaga crenulata* (Froelich) | Polyphagous | Hamilton *et al.* 1971 |
| *Phyllophaga forbesi* (Glasgow) | Polyphagous | Guppy, 1982 |
| *Phyllophaga fusca* (Froelich) | Polyphagous | Guppy, 1982 |
| *Phyllophaga helleri* (Brsk.) | Paddy, sugarcane | Kalshoven, 1981 |
| *Phyllophaga hornii* (Smith) | Polyphagous | Hamilton *et al.* 1971 |
| *Phyllophaga implicita* (Horn) | Polyphagous | Hamilton *et al.* 1971 |
| *Phyllophaga inversa* (Horn) | Polyphagous | Hamilton *et al.* 1971 |
| *Phyllophaga micans* (Knoch) | Polyphagous | Kalshoven, 1981 |
| *Phyllophaga quercus* (Knoch) | Polyphagous | Guppy, 1982 |
| *Phyllophaga rugosa* (Melsheimer) | Polyphagous | Hamilton *et al.* 1971 |
| *Popillia biguttata* (Wied.) | Various flowers | Kalshoven, 1981 |
| *Popillia japonica* (Newman) | Turfgrass | Shelvan *et al.* 1994 |
| *Psilopholis grandis* (Cast) | Hevea, serch | Kalshoven, 1981 |
| *Psilopholis vestita* (Sharp) | Polyphagus | Kalshoven, 1981 |
| *Rhizotrogus majalis* (Razow.) | Vine yard | Kalshoven, 1981 |
| *Sericesthis prunosa* (Dalman) | Polyphagous | Beard, 1956 |
| *Strigoderma arboricola* (Fabricius) | Polyphagous | Hamilton *et al.* 1971 |
| *Strigodermella pygmaea* (Fabricius) | Polyphagous | Hamilton *et al.* 1971 |
| *Xylotrupes gideon* (L.) | Polyphagous | Yadava and Mathur, 1987 |

## Appendix 2.2.2 Natural enemies (predators, parasitoids and pathogens) of white grubs species

| Scientific name | Family/order | Host speciees/stage | References |
|---|---|---|---|
| *Acridotheres tristis* (L.) | Aves | White grubs | Shah and Garg, 1985 |
| *Amara impuncticollis* (Say) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Amara pallipes* (Kirby) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Apocellus sphaericollis* (Say) | Staphylinidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Aspergillus parasiticus* | Hyphomycetes | All stages | Yadav and Mathur, 1987 |
| *Baccillus lentimorbus* (Dutky) | Bacilliaceae | White grubs | Julian and Hall, 1968 |
| *Bacillus cerus* (Frankland ) | Bacilliaceae | White grubs | Yadav and Mathur, 1987 |
| *Bacillus euloomarahae* (Beard) | Bacilliaceae | *H. sanctehelenae* | Steinhaus, 1949 |
| *Bacillus fribourgensis* | Bacilliaceae | *M. melolontha* L. | Franz, 1971 |
| *B. lentimorbus var. australis* | Bacilliaceae | *Sericesthis geminata* | Franz, 1971 |
| *Bacillus popillae* (Dutky) | Bacilliaceae | *P. japonica* Newman | Roberts and Yendol, 1971 |
| *Bacillus popilliae* (Dutky) | Bacilliaceae | *P. japonica* Newman | Julian and Hall, 1968 |
| *Bacillus thruringiensis japonicus* | Bacilliaceae | *Anomala* sp | Zhang *et al.* 1997 |
| *Vagoiavirus melolonthae* | | *M. melolontha* L. | Hurpin and Roberts, 1967 |
| *Baculovirus* | | *S. australis* Boisd. | Kalshoven, 1981 |
| *B. brogniartii* (Sacc.) Petch | Hyphomycetes | *M. melolontha* L. | Keller, 2000 |
| *B. quadrimaculatum* (L.) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Bufo melanosticus* (Schneider) | Bufonidae | Adults and grubs | Yadav and Mathur, 1987 |
| *Caloglyphus sp.* | Acaridae | *P. auxia* (Le Conte) | Jarvis, 1964 |
| *Campsomeris colloris* | Scoliidae | White grubs | Yadav and Mathur, 1987 |
| *Campsomeris annulata* (F.) | Scoliidae | *A. impressicolle* Arrow | Kalshoven, 1981 |
| *Campylocera* sp. | Pyrgotidae | White grubs | Kalshoven, 1981 |
| *Chlaenius tomentosus* (Say) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Chlaenius tricolor* (Dejean) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Clostridium spp.* | | White grubs | Yadav and Mathur, 1987 |
| *Compsomeris llefmansi* (Beten.) | Scoliidae | White grubs | Kalshoven,1981 |
| *Coporoporus laevis* (LeConte) | Staphylinadae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Corvus splendens* (Vieillot) | Aves | White grubs | Shah and Garg, 1999 |
| *Cratacanthus dubius* (Beauvois) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Diplococcus spp.* | | White grubs | Yadav and Mathur, 1987 |
| *Eutrixopsis javana* (Towns) | Tachinidae | White grubs | Kalshoven, 1981 |
| *Evarthrus sodalis* (LeConte) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Gecho gecho* (L.) | Reptile | Adults and grubs | Yadav and Mathur, 1987 |
| *Harpalus caliginosus* (F.) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *H. bacteriophora* (Poinar) | Heterorhabditidae | *M. melolontha* L. | Berner and Schnetter, 2002 |
| *H. bacteriophora* (Poinar) | Heterohabditidae | *P. japonica* Newman | Sulistyanto and Ehlere, 1996 |
| *Megascolia azurea* Chr. | Scolidae | *Xylotrupes gideon* L | Kalshoven, 1981 |
| *M. virginica* (Latreille) | Cicindelidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *M. anisopliae* (Metsch.) Sorokin | Hyphomycetes | White grubs | Steinhaus, 1949 |
| *M. anisopliae* (Metsch.) Sorokin | Hyphomycetes | *X. Gideon* L. | Kalshoven, 1981 |
| *Neohypnus melanops* (Casey.) | Staphylinadae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Ommatius spp.* | Asilidae | | Kalshoven, 1981 |
| *Paenibacillus popillae* | | *M. melolontha* L. | Vestergaard *et al.*1995 |
| *Philodius sp.* | Anoceridae | Eggs and grubs | Kalshoven,1981 |
| *Philonthus cognatus* (Stephens) | Staphylinidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Philonthus varius* (Gyllenhal) | Staphylinidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Prosena sp.* | Tachnidae | White grubs | Kalshoven, 1981 |
| *Rickettsia melolonthae* | | *M. melolontha* L. | Franz, 1971 |
| *S. substriatus* (Haldeman) | Carabidae | *P. japonica* Newman | Terry *et al.* 1993 |
| *Scolia aureipennis* | Scoliidae | White grubs | Yadav and Mathur, 1987 |

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|---|---|---|---|
| *Steinernema glaseri* (Steiner) | Steinernematidae | White grubs | Yeh and Alm, 1995 |
| *S. carocapsae* (Weiser) | Steinernematidae | White grubs | Forschlerand Gardner, 1991 |
| *Steinernema kushidai* (Mamiya) | Steinernematidae | White grubs | Kushida *et al.* 1987 |
| *Tiphia spp.* | Scoliidae | White grubs | Yadav and Mathur, 1987 |
| *Tiphia vernalis* (Rohwer) | Typhiidae | White grubs | Rogers and Potter, 2003 |

**Appendix 2.2.3 Natural enemies (viral diseases) of Scarabaeidae**

| Host species | Virus group | Reference |
|---|---|---|
| *Allomyrina dichotomous* | Iridoviridae | Martignoni and Iwai, 1981 |
| *Amphimallon solstitialis* | Poxviridae | Martignoni and Iwai, 1981 |
| *Anomala cuprea* | Poxviridae | Martignoni and Iwai, 1981; Katagiri *et al.* 1975 |
| *Anoplognathus porosus* | Poxviridae | Martignoni and Iwai, 1981 |
| *Anoxia villosa* | Poxviridae | Martignoni and Iwai, 1981 |
| *Antitrogus morbillosus* | Poxviridae | Martignoni and Iwai, 1981 |
| *Aphodius tasmaniae* | Poxviridae, Nodaviridae | Martignoni and Iwai, 1981; Goodwin and Filshie, 1975 |
| *Costelytra zealandica* | Irioviridae, Nodaviridae | Martignoni and Iwai, 1981 |
| *Dasygnathus* sp. | Poxviridae | Martignoni and Iwai, 1981 |
| *Demodena boranensis* | Poxviridae | Martignoni and Iwai, 1981 |
| *Dermolepida albo-hirtum* | Poxviridae | Vago *et al.* 1968 |
| *Geotrupes silvaticus* | Poxviridae | Martignoni and Iwai, 1981; Goodwin *et al.*1991 |
| *G. stercorosus* | Poxviridae | |
| *Heteronychus arator* | Iridoviridae | Martignoni and Iwai, 1981 |
| | Group C Baculoviridae | Martignoni and Iwai, 1981 |
| *Hoplia* sp. | Poxviridae | Martignoni and Iwai, 1981 |
| *Melolontha melolontha* | Parvoviridae, Poxviridae | Martignoni and Iwai, 1981; Goodwin *et al.*1991 |
| *Odontria* sp. | Iridoviridae | Martignoni and Iwai, 1981; Moore *et al.* 1974 |
| *Opogonia* sp. | Iridoviridae | Martignoni and Iwai, 1981 |
| *Oryctes boas* | Group C Baculoviridae | Martignoni and Iwai, 1981 |
| *O. monoceros* | Group C Baculoviridae | Martignoni and Iwai, 1981 |
| *O. nasicornis* | Group C Baculoviridae | Martignoni and Iwai, 1981 |
| *Othnonius batesi* | Proxviridae | Martignoni and Iwai, 1981 |
| *Papuana uninodis* | Group C Baculoviridae | Zelazny *et al.* 1988 |
| *Pericoptus truncatus* | Nodaviridae # | Martignoni and Iwai, 1981 |
| *Phyllopertha horticola* | Proxviridae | Martignoni and Iwai, 1981 |
| *Phyllophaga anxia* | Iridoviridae | Poprawski and Yule, 1990 |
| *P. pleei* | Proxviridae | Martignoni and Iwai, 1981 |
| *Proagopertha lucidula* | Proxviridae | Goodwin *et al.*1991 |
| *Poillia japonica* | Iridoviridae | Goodwin *et al.*1991 |
| *Rhopaea verraxi* | Proxviridae | Martignoni and Iwai, 1981 |
| *Sericesthis pruinosa* | Group C Baculoviridae | Martignoni and Iwai, 1981 |
| *S. nigrolineata* | Proxviridae | Milner and Lutton, 1975 |
| *Strategus aloeus* | Group C Baculoviridae | Lomer, 1987 |

# Chapter 3

# Exploratory study towards microbial control of white grubs in Nepal



This chapter is based on a paper published in the Bull. Soc. Ent. Suisse 76, 249-258 (Yubak G. C. and S. Keller, 2003)

# CHAPTER 3

## Exploratory study towards microbial control of white grubs in Nepal

### 3.1 SUMMARY

With an objective to explore the possibility of biocontrol of white grubs using entomopathogenic fungi, an exploratory study was conducted in the Parbat, Syangja, Tanahun and Chitwan Districts of Nepal since the mid of 2001/2002. In order to explore the occurrence of indigenous fungal pathogens of white grubs, field and laboratory experiments were carried out and information were collected from all available sources. Upon collection of the white grubs the entomopathogenic fungus *Metarhizium anisopliae* (green muscardine fungus) and *Beauveria bassisana* (white muscardine fungus) were found to be associated with white grubs and soils in fields with arable crop. Disease prevalence with *M. anisopliae* was between 0 and 2% depending on host origin and species and *B. bassiana* was found only from few soil samples. Bioassays with *M. anisopliae* revealed that the Nepalese isolates of this fungus species were as pathogenic as a Swiss isolate used for comparison purposes. Therefore, future work will be done exclusively with Nepalese isolates. Analysis of soils from three different regions showed that *M. anisopliae* is common and was present in about 50% of the samples irrespective of their origin. However, the fungus densities were low. Based on these first results, the possibilities to develop mycoinsecticides and to integrate them into existing pest management (IPM) systems are considered as very promising.

### 3.2 INTRODUCTION

Nepal represented by the Terai (from below 100 m asl) to the highest peak of the world (Mt. Everest, 8, 848 m asl) has a wide variety of climatic and agro-ecological zonations, where agriculture is the main source of livelihood. The crops grown are also very diverse, from tropical rice to temperate barley, from mangoes to apples. Agriculture in the past was of traditional with few crops per unit land per year, low input in terms of chemicals (fertilisers and pesticides) resulting in low productivity. With increasing population pressure, the need for the adoption of improved technologies has been widely realised at all levels from planners to rural farmers. Adoption of new technologies has lead to the adoption of high yielding varieties and high input of fertilisers and pesticides. These practices have lead not only to increased production and productivity and greater monetary returns to the farmers but also resulted in severe problems to an extent never faced in the past. Pest problems including insects, diseases, weeds, rodents, and mites have increased greatly in several crops. The National Planning Commission of Nepal has estimated that pests cause a loss in food production of about 15-20% (Baker and Gyawali, 1994). Farmers are very vigilant and worried about pest attack in high value crops such as rice and maize and most vegetables and fruits. In such crops they often apply pesticides to reduce the damage to the crops in an excessive way.

Different types of insect pests attack different crops; however, the losses in cereal are one of the major concerns for the farmers and technicians as they are major sources of livelihood in Nepal. Crop losses by soil-pest insects have been identified as one of the main causes of low food production. Among different insect pests, white grubs (Coleoptera:

Scarabaeidae*)* are among the destructive insect pests in Nepal. Collaborating Institutions of Sustainable Soil Management Program (SSMP) have reported white grubs as a major limiting factor to soil productivity in several areas. White grubs, for example, caused an average of about 25% yield loss on groundnut in Baitadi District of Nepal (G. Weber, pers. comm.). In Nepal losses due to white grubs are reported from across the country of the mid to high hills, although quantification of their damage has not been assessed. In Himachal Pradesh, India, potato yield losses of up to 85% have been reported (Misra and Chandla, 1989).

White grubs are not new pest insects in Nepal, however, in farmers' perception their infestation has been increasing every year. A hitherto unknown number of species of white grub causes damage depending mainly on environmental conditions; however, the damaging species are not identified yet. They are polyphagous and feed on roots, which may lead to the complete destruction of single plants, or of the entire plant cover in a limited area. Their life cycle is species specific and may last one or more seasons. The adults are considered less damaging but some species are known to feed on roots, leaves, buds and flowers of cultivated plants. Traditional methods to control white grubs are repeated ploughing, preferably soon after the summer rains, which kills them or expose them to predatory animals, removal of host weeds by cleaning nearby areas, the use of well decomposed manure, and an adapted crop rotation. As a control measure, farmers attempt different methods, however, the pests are not amenable to control with the common practices. In recent years, chemical insecticides including DDT were more and more used to control this pest. In order to avoid detrimental effects of such a practice, the SSMP funded by Swiss development organizations (SDC, Helvetas, Interco operation) initiated a project with the goal to develop and establish a biological control method using indigenous antagonists. Such methods have been developed and successfully applied in other countries like Switzerland, Austria, New Zealand and Australia (Keller *et al.* 2000; Strasser, 1999; Jackson *et al.* 1992; Rath *et al.* 1995). They were either based on the insect pathogenic bacteria *Serratia entomophila* (Jackson *et al.* 1992) or the insect pathogenic fungi *Metarhizium anisopliae* (Zimmermann, 1993) and *Beauveria brongniartii* (Zimmermann, 1992).

## 3.3 MATERIAL AND METHODS

### 3.3.1 White grub problems and farm management

During the visit information related to severe-ness of white grub's problem, crop attacked and their damage (Figure 3.1) period and prevailing control practices were also collected. At the same time, information regarding the use of chemical pesticides and possibility of exploring alternative measures were collected. Based on the information of damages due to white grubs, a survey was carried out in the farming sites of Parbat, Syangja, Tanahun and Chitwan (Figure 3.2).

Figure 3.1 White grubs damage in vegetable crops (left) and adult *Heteronychus lioderes* attacking maize seedlings (right)


Figure 3.2 Study sites of the collection of insect pathogenic fungi in Nepal (1 Durlung and Pang/Parbat; 2: Syangja; 3: Rishing Patan/Tanahun; 4: IAAS and NMRP Rampur/Chitwan. White grubs were collected from 1 and 2; and soil samples were collected from 1, 3 and 4).

### 3.3.2 Collection of white grubs

Soil samples and fresh living white grubs were collected from the surveyed area. They were further analyzed for the natural presence of the fungus at the Insect Pathology laboratory of IAAS, Rampur, Nepal and at the laboratory of Agroscope FAL, Reckenholz, Switzerland. White grubs were collected (Figure 3.3) from plots of 25 $m^2$ of white grub prone areas of Bari land in two ecological zones of low-mid hills (600-900 m asl) in Syangja and mid-hills (1200-1600 m asl) of Parbat Districts.

Figure 3.3 Collection of white grubs in farmer's field

The sampling in these two zones was aimed at collecting different species of white grubs and different races of fungal pathogens attacking them.

A total of three farms were identified. Samples were taken from fields with high infestation by white grubs in recent cropping season, fields with a high infestation some 2-3 years ago and fields with significant decline in infestation over the past two years (possibility of decline because of fungal pathogens). The field identification was cross-checked with several farmers and the purpose of identifying fields of high and declining infestation was explained. A total of 600 white grubs (300 grubs per District having 50 larger types, 200 smaller types and 50 other types).

Immediately after the collection of white grubs, they were placed in containers with soil from the same collection site for transporting them to the laboratory. Only 5 larvae were put into each container and food items such as slices of carrot and potato were added so as to avoid cannibalism. Additional soil (15 kg) was collected from the same field for the laboratory rearing under the natural white grub habitat. While surveying, some of the basic information such as name and address of the farm, ecological conditions of the farm (altitude, slope aspect, soil texture, soil organic matter content estimate, soil pH, soil microbial activity), history of cropping over the past 3 years, field management (frequency of ploughing, FYM application), history of white grub attack over the previous seasons, 1 year ago, 5 years ago, 10 years ago and farmers knowledge about what causes an increase or a decrease in white grub problems were recorded. Detailed information regarding the survey is presented in Table 3.1.

### 3.3.3 Rearing of white grubs

Rearing of the white grubs (Figure 3.4) was continued in the captivity at the Entomology laboratory (Insect Pathology Unit) of the TU, IAAS, Rampur. In the laboratory, the larvae were separated singly into individual poly pots (4.5 cm diameter and 6 cm height) and fed with the slices of carrot, potato, tree yam and ground nuts. Soils collected from the original sampling farms were poured into the containers to provide natural habitat to the grubs.

Figure 3.4 Rearing of white grubs at IAAS, Rampur

A separate coding system was developed and applied to the individual rearing pots indicating the sampling sites and grubs intensity. The sampling sites were chosen based on farmers report as white grubs affected area in Syangja (Dhanubanse) and Parbat (Pang) Districts of Western Nepal. The rearing room was protected from direct sun-light and UV-light by making it dark. The rearing temperature was within the range of 22-23 $^{o}$C during the rearing period. Rearing vials were covered with muslin cloth and tied with rubber bands to ensure good aeration. Moisture was maintained through wet cloth and soil with gentle moistening when the soil felt dry.

Artificial diets such as carrot, potato, groundnut, tree yam were provided ad-libitum and changed every five days. Food and environmental stress were avoided during rearing of the grubs. While rearing of the grubs, the following biological parameters such as, date of collection, date and causes of grub mortality, date of pupation and adult emergence were recorded.

### 3.3.4 Observation of insect pathogenic fungi from white grub

The larvae were reared individually in poly vials in dark condition. All the larvae kept for rearing were checked daily until the emergence of adults or their death whether they are attacked by the insect pathogenic fungi. Dead larvae were carefully separated and transferred into another vial with cotton in order to assess the causes of mortality either due to fungus or any other reasons.

Figure 3.5 White grubs succumbed with *Metarhizium anisopliae*

Larvae suspected to be affected by fungi (Figure 3.5) were kept at humid and damp condition to enhance the fungus sporulation. The larvae which did not show any fungal symptoms were kept individually in incubator (1-5 $^{o}$C) identification and checked later for presence of fungi. Pupae were left in the same rearing containers along with the soil and earthen cocoons for the observation of adult emergence and identification of the species. Before isolation, confirmation of the attack by *Metarhizium anisopliae* was checked under the light microscope as shown in the Figure 3.6.

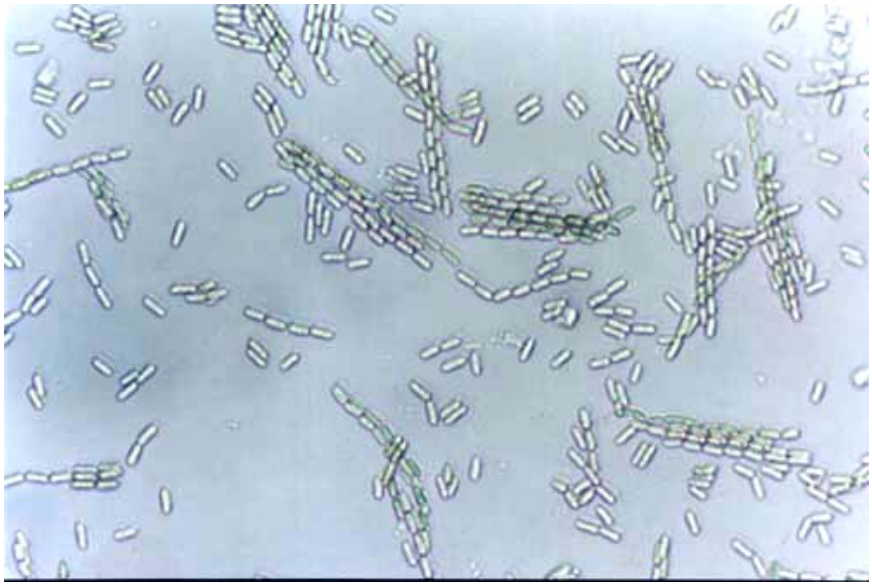
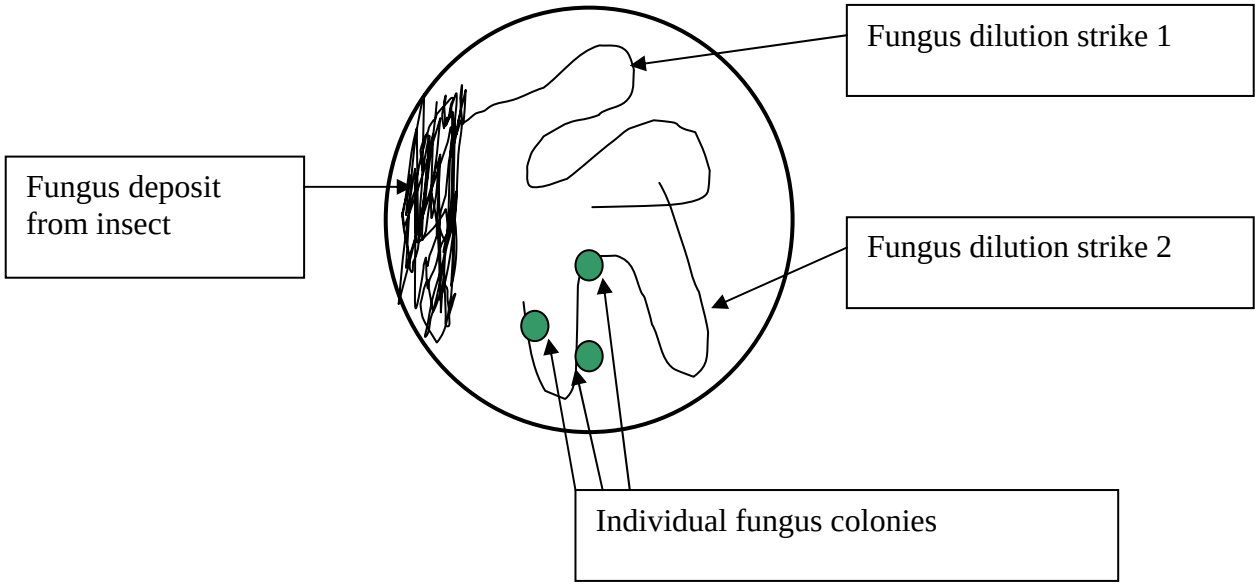


Figure 3.6 Conidia of *Metarhizium anisopliae* under light microscope

Isolation of the fungus was carried out following the loop dilution method (Figure 3.7) from such cadavers that were fully covered by the sporulating fungus.




Figures 3.7 Isolation of fungus with loop dilution method (up) and growth of conidia (below)

### 3.3.5 Observation of insect pathogenic fungi from soils

Forty six soil samples were taken with a soil core from the farmer's fields of Parbat, Syangja, Tanahun and Chitwan Districts. All samples were checked with the Galleria bait method (GBM) (Zimmermann, 1986) (Figure 3.8).

Figures 3.8 Galleria bait method (GBM) at the day of setting (left) and turning upside down (right) during baiting

They originated from arable land and from meadows in Chitwan (Rampur, National Maize Research Programme – NMRP), from Syangja (Dhanubanse), from Tanahun (Rising Patan) and Parbat (Pang, Durlung) and from fruit plantations, arable land and meadows from Chitwan (Rampur, Tribhuvan University, Institute of Agricultural and Animal Sciences – IAAS).

Thirty soil samples, ten each from Rising Patan, Pang, and NMRP were analyzed by plating soil suspension on a selective medium (Keller et al. 2000). The GBM was adapted from Zimmermann (1986). About 60 ml of soil/sample was filled in a cylindrical plastic vials (4.5 cm diameter and 6 cm height) and 4 larger *Galleria* larvae added. The samples were kept in darkness at a temperature of 22 $^{o}$C. During the first five days the tubes were turned (Figure 3.7, right) daily to keep the larvae moving in the soil. After 16-18 days the larvae were examined, fungus infections were recorded and the fungus from infected larvae was isolated.

### 3.3.6 Isolation of soil fungi with the soil suspension method

The soil plating method was adapted from Fornallaz (1992) (Figure 3.9). 10 g soil/sample of fresh soil are shaken for 3 h at 140 rpm on a rotary shaker in 250 ml Erlenmeyer flasks with 50 ml tap water containing 1.8 g / l tetra - Sodiumdiphosphate-Decahydrat ($Na_4P_2O_7$*$10H_2O$) to favour disaggregation of the soil. After 15 seconds of sedimentation 0.1 ml the suspension is distributed with a Trigalsky spatula on a Petri dish with selective medium and intensively rubbed. Three replicates/soil samples were prepared. After 8-10 days at 20 $^{o}$C and darkness the colonies of *M. anisopliae* were counted and a selection of colonies was isolated in tubes with Sabouraud-glucose-agar with 0.6 g/l Streptomycin. The soil suspension was not diluted, even if there were very high fungal densities exceeding 600 colonies Petri dishes, the maximum that could be counted.

Figure 3.9 Isolation of fungus from soil (soil shaking method)

The selective medium adapted from Strasser *et al.* (1997) with the following composition and preparation was used: 10 g Peptone from meat pancreatically digested, 20 g Glucose, 18 g Agar-agar, all dissolved in 1 l distilled water and autoclaved at 120 $^{o}$C for 20 minutes. At a temperature of 60 $^{o}$C; 0.6 g Streptomycin, 0.05 g Tetracycline and 0.05 g Cyclohexamide previously dissolved in distilled, sterile water and 0.1 ml Dodine were added.

## 3.4 RESULTS

### 3.4.1 White grub problems and farm management

The majority of the farmers were unaware of the increasing problem of white grubs, their nature and stages of damage and appropriate control measures. In the surveyed area, the farmers ignored crop management such as cultural practices, use of resistant varieties, fertilizer and irrigation management. It turned out that the farmers had no knowledge about the use of decomposed farmyard manure (FYM), mixed cropping, crop rotation and other adequate practices.

### 3.4.2 Use of chemical pesticides

In recent years, pesticide use in Nepal has increased significantly, and this trend is likely to continue and even accelerate in the near future due to missing alternatives. The area along the open boarder with India where agro-chemical production is growing without much quality control is particularly vulnerable. However, in the surveyed area, 25-30% of the farmers used different types of pesticides such as DDT, benzene-hexachloride (BHC), other poor graded dusts and chlorpyrifos to control white grubs, however, the percentage is much bigger in case of off-season vegetable crops. During survey, it was also learnt that few

farmers also use fungicides such as Ridomil in case of Parbat site because of the wrong perception.

The most alarming aspect is that the majority of the farmers cannot read the labels on the pesticide packages or containers and often rely on verbal instructions of the retailer to use pesticide. There is still great misconception among some farmers as they think pesticides are less dangerous chemical compounds. Because of this, there is high level of negligence and misuse while handling chemical pesticides. Moreover, most farmers are not aware of the hazards related to chemicals and do not have adequate knowledge of safety measures.

### 3.4.3 Association of entomopathogenic fungi in white grubs and soils

The white grubs belonged to different and not completely identified Scarabaeid species. They were collected at twenty four locations in two Districts and separated into three groups: "large larvae", later on identified as *Xylotrupes gideon*, "small larvae" later identified as *Maladera affinis* and "other larvae" belonged to *Anomala, Pyhllophaga, Holotrichia* etc. The disease prevalence varied between 0% and 2% infected larvae. Infections were found only in the group of "small larvae" (Table 3.1). All entomopathogenic fungi isolated from grub cadavers were identified as *Metarhizium anisopliae*.

Table 3.1 Natural occurrence of entomopathogenic fungi on white grubs in 2002 at IAAS, Rampur, Chitwan, Nepal.

| Origin of white grubs | White grub species | Number of white grubs observed | Number of white grubs infected with fungus | % fungus infected grubs |
|---|---|---|---|---|
| Syangja (Mid hill, Western region) | *Xylotrupes gideon* ("Large grubs") | 50 | 0 | 0 |
| | Small grubs | 200 | 0 | 0 |
| | Others | 50 | 0 | 0 |
| Parbat (Mid hill, Western region) | *Xylotrupes gideon* ("Large grubs") | 50 | 0 | 0 |
| | Small grubs | 200 | 4 | 2 |
| | Others | 50 | 0 | 0 |

*M. anisopliae* was found at all locations and in all types of crop except in arable land at Tanahun. *Beauveria bassiana* was found in grassland at Parbat (Pang), Tanahun (Rishing Patan), and in arable land at Tanahun (Rishing Patan) and Chitwan (NMRP, Rampur) (Table 3.2).

Two fungus specimens attributed to *Paecilomyces* spp were found in grassland at Tanahun. Two methods were used to study the presence of entomopathogenic fungi in soils: the GBM and spreading soil suspensions on selective media. Using the GBM 52% of the soil samples proved to contain entomopathogenic fungi. The difference between soils from arable land (48% positive samples) and soils from grassland (57% positive samples) were only small and statistically not significant ($p = 0.73$).

Table 3.2 Presence and density of entomopathogenic fungi (EPF) in soil samples from two sites in Nepal as pointed out with the *Galleria* bait method in 2002 at IAAS, Rampur, Chitwan, Nepal. *M.a.* = *Metarhizium anisopliae; B. bass.* = *Beauveria bassiana*; *P.* sp. = *Paecilomyces* sp. NMRP = National Maize Research Programme

| Origin | Crop | Number of samples | Soil samples with EPF (%) | Species of EPF |
|---|---|---|---|---|
| Parbat, | Arable land | 4 | 3 (75) | 3 *M.a.* |
| Durlung | Grassland | 4 | 2 (50) | 2 *M.a.* |
| Parbat, Pang | Arable land | 5 | 3 (60) | 3 *M.a.* |
| | Grassland | 5 | 3 (60) | 3 *M.a.* + 1 *B. bass.* |
| Tanahun, | Arable land | 5 | 1 (20) | 1 *B. bass*. |
| Rishing Patan | Grassland | 5 | 4 (80) | 1 *M.a.* + 4 *B. bass*. + 2 *P.* spp. |
| IAAS, Rampur | Arable land Fruit plantations | 4 | 2 (50) | 2 *M.a.* |
| | Grassland | 4 | 1 (25) | 1 *M.a.* |
| NMRP, | Arable land | 5 | 2 (40) | 2 *M.a.* + 1 *B. bass*. |
| Rampur | Grassland | 5 | 3 (60) | 3 *M.a.* |
| All | Arable land | 23 | 11 (48) | 10 *M.a*. + 2 *B. bass*. |
| | Grassland | 23 | 13 (57) | 10 *M.a*. + 5 *B. bass*. |

Ten soil samples from three localities each were analyzed for the density of *M. anisopliae* (Table 3.3). This fungus was found in two samples from Parbat (Pang), in one sample from Tanahun and in two samples from Rampur (NMRP). The highest density found was 275 CFU/g fresh soils. However, these data are not representative since all three replicates of 15 out of 30 samples were overgrown with fast growing fungi and the presence of *M. anisopliae* could no more be checked.

Table 3.3 Presence and density of entomopathogenic fungi (EPF) in soil samples from two sites in selective medium in 2002 at IAAS, Rampur, Chitwan, Nepal (SM: Selective Medium. CFU = Colony Forming Units. *M.a.* = *Metarhizium anisopliae*).

| Origin | Crop | Number of samples | Soil samples with EPF (%) | SM CFU/g fresh soil | Species of EPF |
|---|---|---|---|---|---|
| Parbat Pang | Arable land | 5 | 1 (20) | 275 | *M.a.* |
| | Grassland | 5 | 1 (20) | 35 | *M.a.* |
| Tanahun | Arable land | 5 | 0 | | |
| Rishing | Grassland | 5 | 1 (20) | 15 | |
| Patan | | | | | |
| Rampur | Arable land | 5 | 0 | | |
| NMRP | Grassland | 5 | 2 (40) | 265* | *M.a.* |
| all | | 30 | 5 (17) | | |

* Data from a single sample only, the other sample was partially overgrown by saprophytic fungi.

## 3.5 DISCUSSION

Increasing damages in different kind of farm crops due to larvae of Scarabaeidae (white grubs) have drawn attention to this group of root feeding pest insects. First surveys revealed crop losses of 25% and white grubs pointed out to be among the most important pest

insects in Nepal. However, damages result not only in crop loss, the feeding on grass roots may cause instability and collapses of terraces and loss of arable land or the possibility to irrigate the crops. In many regions the farmers became aware of the white grub problem, but no traditional pest management practice turned out to be effective. As a consequence, more and more synthetic insecticides including the persistent organochlorines are applied although they are banned for agricultural use. Some of them have no effects on white grubs, but have well documented impacts on the environment. This development alarmed agricultural authorities and lead to the initiation of a project for the biological control of white grubs.

Several workers have reported a number of biological control agents, which attack white grubs in different parts of the world. The development of commercial products based on entomopathogenic fungi for the use in integrated pest management programmes needs several steps. Fungal species and isolates must first be obtained from diseased insects or from the environment, and identified. The most promising candidates are evaluated under laboratory conditions and then produced in large scale as mycopesticides.

## 3.6 CONCLUSIONS

Further the large number of species of Scarabaeidae causing damages in Nepal is not yet identified. Research in this direction was initiated in summer 2002 together with a survey on the presence of entomopathogenic fungi attacking white grubs.

The study has revealed low natural prevalence of fungus diseases. Depending on species and origin, a maximum on 2% of the grubs was infected with *M. anisopliae*. Other pathogenic fungi were not found. Analysis of soil samples taken in four regions showed, the presence of other EPF especially *M. anisopliae* proved to common at all places. The result demonstrated that the GBM was much more efficient and appropriate to analyze soils for the presence of entomopathogenic fungi. In addition, this method is simple and allows finding of other species of entomopathogenic fungi. Therefore, it is recommended to use it in future investigations, either in surveys for entomopathogenic soil fungi or in monitoring the fungus distribution in control trials. The poor results of the isolation with selective medium can not be attributed to the method itself. It is attributed to the characteristic of the soils that contain high densities of saprophytic fungi, which rapidly overgrew the medium. In the experiment all three replicates of 15 out of 30 samples could not be analyzed due to heavy contaminations. The problem is also known from Swiss soils, where an average of 5-10% of the Petri plates can not be analyzed. However, it is very rare that all replicates of a sample are that heavily contaminated. In Switzerland comparable results are obtained with either method (Keller *et al.* 2003).

The percentage of soil samples containing entomopathogenic fungi as well as the densities found are low as compared to data from Switzerland, but are in the range of other European countries. In Switzerland 96% of the fields analyzed were found to contain *M. anisopliae* with an mean density of about 1000 CFU/g dry soil (Keller *et al.* 2003), in Germany and Norway 60% and 7.5%, respectively, of the soil samples contained entomopathogenic fungi dominated by *M. anisopliae* (Kleespies *et al.* 1989; Klingen, 2000).

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# Chapter 4

## Preliminary screening of various isolates of the entomopathogenic fungus, *Metarhizium anisopliae* against white grubs through bioassay experiment



Part of these results has been published in Proceedings of Fourth National Conference on Royal Nepal Academy of Science and Technology (RONAST), Kathmandu, Nepal (Yubak Dhoj G. C. *et al.* 2004).

# Chapter 4

## Preliminary screening of various isolates of the entomopathogenic fungus, *Metarhizium anisopliae* against white grubs through bioassay experiment

### 4.1 SUMMARY

White grubs are increasingly important pests of cereal and cash crops in Nepal. The situation is also aggravated by the fact that they are one of the most difficult insect pests to manage due to high levels of insecticide resistance (Baker and Gyawali, 1994). Series of activities with respect to screening were conducted since the middle of 2003 at the Institute of Agriculture and Animal Sciences (IAAS), Rampur of Tribhuvan University (TU) and in farmers' fields in Nepal. Since exploratory study, several dozens of isolates of the insect pathogenic fungi, *Metarhizium anisopliae* (green muscardine fungus) and about half a dozen of *Beauveria bassiana* (white muscardine fungus) were isolated from infested soil and diseased insects using a selective medium and the *Galleria* bait method (GBM). Isolation, maintenance, mass production and efficacy tests with *M. anisopliae* were conducted under captivity and under field condition. A three-tired screening strategy was adopted to evaluate the pathogenicity of *M. anisopliae* through bioassay. Time mortality studies with concentrations of $10^7$ spores /ml indicated that eight isolates gave over 80% infected grubs, 65 isolates gave over 50-60% infected grubs and rest of the isolates had a low pathogenicity. Five of them, M1, M6, M18, M48, and M50, were highly pathogenic ($p<0.001$) against third instar larvae of *Maladera affinis* Blanchard. The LT50 of the tested isolates varied between 2-9 weeks, 12 isolates were highly virulent with an LT50 of 2-4 weeks, 34 isolates had a moderate virulence with an LT 50 of 5-6 weeks and 22 isolates had a low virulence. The isolates M1 and M6 had the highest mortality rates and the shortest time to kill the larvae. These virulent isolates were promoted for further studies of mass production and field application.

### 4.2 INTRODUCTION

White grubs are economically important pest insects in Nepal, however, its strategic management is lacking in the country (G. C. and Keller, 2002). Microbial control exploits the disease causing organisms to reduce the population of insect pests below the damaging level (Dhaliwal and Arora, 2002). Epizootics of the entomopathogenic fungi *Metarhizium anisopliae* is reported from several scarab species and other soil inhibiting Coleoptera (Fleming, 1968; Hurpin and Robert, 1972; Young, 1974; Glare, 1992). Susceptibility is often a quantitative phenomenon, with high dosages of $10^8$ to $10^9$ conidia per ml causes normally the higher mortality (Glare and Milner, 1991). Very scattered and accidental uses of microbial pesticides have been reported in some pocket areas of Nepal, however, none of them are obtained from indigenous sources. They are produced in other countries and used basically with the research interest. In this sense, the use of indigenous biocontrol agents (BCAs) is entirely a newer approach in the country. Exploratory study conducted by Tribhuvan University, Institute of Agriculture and Animal Sciences (IAAS), Rampur in the farmer's fields has indicated ample opportunity of working with native species of the insect pathogenic fungi as a means to control white grubs in particular and soil insect pests in

general. Realizing the need to reduce the use of health hazardous chemicals, a project on indigenous BCAs to control white grubs is initiated.

Entomogenous fungi have great promise to be uses as BCAs against different insects; however, their infectivity is quite different depending on fungus species and developmental stage of the insects (Samson, 1981). Therefore, when a particular insect pest control programme is considered using these fungi, the particular species or strains which are most suitable have to be taken into account. Similarly, the time of exposure of the host to insect pathogenic fungi and the time taken to kill the host are also important parameters for evaluating the virulence of these fungi. Fungi which need a short exposure period and kill the host fast are very important in the practical application. Therefore, it is important that the activity of selected fungus isolates should be screened against the particular target host at the initial stage. Such pathogenic relationships may give ideas related to virulence of the species and the amount of fungus spores necessary to kill at least 50% or more insect pests. Some laboratory experiments were conducted to study the efficacy of selected isolates of entomopathogenic soil fungi (EPSF) against the white grubs.

## 4.3 MATERIAL AND METHODS

### 4.3.1 Collection and maintenance of fungus strains

In order to obtain isolates of the (EPSF), soil samples and white grubs were collected from different cropping sites of Parbat, Tanahun, Chitwan and Nawalparasi Districts of Nepal. Insect pathogenic fungi based on *M. anisopliae* (green muscardine fungus) and *B. bassiana* (white muscardine fungus) were isolated from white grubs' cadavers, *Galleria* bait method (GBM) and selective medium through soil dilution plating. These fungi were identified by the expert Dr Siegfried Keller, Agroscope FAL Reckenholz, Zurich, Switzerland based on their conidial sizes and shapes. The fungus strains were isolated and maintained on selective medium at IAAS, Rampur, Chitwan as described in Chapter 3.

### 4.3.2 Production of fungal conidia

The fungi were produced on selective medium with the following composition and preparation: 20 g Glucose, 10 g Peptone, 18 g Agar-agar all dissolved in 1 l distilled water and autoclaved at 120 $^{o}$C for 20 minutes. At a temperature of 60 $^{o}$C; 0.6 g Streptomycin, 0.05 g Tetracycline and 0.05 g Cyclohexamide were added in the same solution. The sterile SDA was transferred into sterialized Petriplates and test tubes. The fungi were incubated at 27±2 $^{o}$C, 80±5 % RH to induce growth and sporulation of the fungus. After 12-15 days, the conidia were harvested by scrapping off the contents from each Petri dish as well from the test tube with the help of sterile bacteriological loop.

### 4.3.3 Maintenance and enumeration of fungal conidia

In order to avoid the loss of virulence of the isolates, regular host passage was undertaken using white grubs. It was performed after each third successive sub-culturing on the selective medium (Fargues and Robert, 1983). The fungus cultures were stored in a refrigerator at 4-5 $^{o}$C until their further use. The fungus isolates were grown on selective medium (Strasser *et al.* 1996). After 12-15 days of incubation the conidia were harvested

either by scrapping off with a loop or by washing off with 0.1% Tween 80. The conidia were suspended in 0.1% Tween 80 and adjusted to $10^7$ spores /ml using Thoma haemocytometer. Detailed methodology of calculation of the fungus spore suspension is presented in Appendix 4.1.

### 4.3.4 Susceptibility study through bioassay experiment

In all cases, conidia were used with a single dose ($10^7$ spores /ml) originating from fungus culture not older than twenty one days. In order to determine the most virulent strain, initially the efficacy of the entire seventy different isolates (Appendix 4.1) was tested against third instars larvae. The white grubs used in the experiments were collected from the damaging sites of Chitwan and identified later. To identify the species, some of the larvae were preserved in 99% alcohol after boiling them in hot water and some were reared to adults for later identification.

The experiment was arranged in a completely randomized design (CRD). The treatments were allotted randomly using random numbers selected from random number table (Gomez and Gomez, 1984). The experimental unit consisted of poly pots and sterile soils. The soil used in the experiment was sterilized in an autoclave at 120 $^{o}$ C and 15 lbs pressure. The soil was cooled and used for grub rearing for bioassay test. The larvae were quarantined in the laboratory for 6 weeks before they were used in the experiment. For each strain 30 white grubs of the third instar were dipped into the spore suspension for five seconds (Goettel and Inglis, 1997) by holding them at the leg with loose forceps. Excess liquid was dropped off and the larvae were placed individually in 100 ml plastic vials (4.5 cm diameter and 6 cm height) half filled with sterile soil and placed in the experimental room at a temperature of 22-24 $^{o}$C. The lid was perforated for air circulation. One control was left untreated; the larvae of the other control were dipped in water. The larvae were fed with slices of potatoes and checked for mortality every third day for ten weeks. Dead larvae were investigated under the stereomicroscope for fungal infection. Mortality and infection rate was calculated using the following formula and virulent strains were determined based on LT 50.

**Following parameters were calculated using following formula;**

1. Mortality % = $\frac{\text{No. of dead insects}}{30} \times 100$
2. Infection % = $\frac{\text{No. of infected insects}}{30} \times 100$
3. LT 50 = Lethal time of 50% of the population due to *Metarhizium anisopliae* was calculated with a probit analysis and expressed in weeks.

Recorded parameters were analyzed in MSTAT-C computer package. Analysis of variance (ANOVA) was performed to observe the percent mortality and infection.

### 4.3.5 Temperature and humidity

The daily maximum and minimum experimental room temperature and humidity was recorded by using BEURER, HM11 Art-Nr.: 678.01 CE instruments which are presented in the Appendix 4.2.

## 4.4 RESULTS

### 4.4.1 Collection and maintenance of fungus strains

Since the mid of 2002, seventy different strains of *M. anisopliae* and eight strains of *B. bassiana* were recovered from white grubs and from soil samples. A copy of each isolates is stored as security copy at the Agroscope FAL Reckenholz, Switzerland with their local code. Detailed information pertaining to fungal isolates are summarised in Table 4.1.

Table 4.1 Origin of the insect fungus from different sites of Nepal in 2003/04. (GBM = *Galleria* Bait Method; SM = Selective medium M1-M52 = *Metarhizium anisopliae* isolates; FAL = Federal Research Station for Agro ecology and Agriculture, Zurich, Switzerland; IAAS = Institute of Agriculture and Animal Sciences).

| Fungus species | Origin | | Date of isolation | Name of the isolator | Remarks | Coding of the strains | |
|---|---|---|---|---|---|---|---|
| | Host | Locality | | | | FAL | IAAS |
| *M. anisopliae* | White grub | Parbat/Nepal | 31.5.02 | S. Keller | Cropland | 800 | M1 |
| *M. anisopliae* | White grub | Parbat/Nepal | 12.6.02 | Y. D. GC | Cropland | | M2 |
| *M. anisopliae* | White grubs | Parbat/Nepal | 12.6.02 | Y. D. GC | crop land | | M3 |
| *M. anisopliae* | Soil/SM | Parbat/Nepal | 31.5.02 | S. Keller | grassland | 801 | M4 |
| *M. anisopliae* | Soil/SM | Parbat/Nepal | 31.5.02 | S. Keller | cropland | 802 | M5 |
| *M. anisopliae* | Soil/SM | Parbat/Nepal | 31.5.02 | S. Keller | cropland | 803 | M6 |
| *M. anisopliae* | Soil/Galleria | Rampur/Nepal | 03.7.02 | S. Keller | cropland | 804 | M7 |
| *M. anisopliae* | Soil/Galleria | Rampur/Nepal | 03.7.02 | S. Keller | cropland | 805 | M8 |
| *M. anisopliae* | Soil/Galleria | Rampur/Nepal | 03.7.02 | S. Keller | grassland | 806 | M9 |
| *M. anisopliae* | Soil/Galleria | Parbat/Durlong | 03.7.02 | S. Keller | cropland | 807 | M10 |
| *M. anisopliae* | Soil/Galleria | Parbat/Durlong | 03.7.02 | S. Keller | cropland | 808 | M11 |
| *M. anisopliae* | Soil/Galleria | Parbat/Durlong | 03.7.02 | S. Keller | cropland | 809 | M12 |
| *M. anisopliae* | Soil/Galleria | Parbat/Durlong | 03.7.02 | S. Keller | grassland | 810 | M13 |
| *M. anisopliae* | Soil/Galleria | Parbat/Durlong | 03.7.02 | S. Keller | grassland | 811 | M14 |
| *M. anisopliae* | Soil/Galleria | Parbat/Pang | 12.9.02 | Y.D. GC | crop land | | M15 |
| *M. anisopliae* | Soil/Galleria | Parbat/Pang | 12.9.02 | Y.D. GC | crop land | | M16 |
| *M. anisopliae* | Soil/Galleria | Parbat/Pang | 12.9.02 | Y.D. GC | Cropland | | M17 |
| *M. anisopliae* | White grub | Parbat/Pang | 01.2.03 | Y.D. GC | Cropland | 885 | M18 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 886 | M19 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 887 | M20 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 888 | M21 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 889 | M22 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 890 | M23 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 891 | M24 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 892 | M25 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Grassland | 893 | M26 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Grassland | 894 | M27 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 895 | M28 |
| *M. anisopliae* | GBM | NMRP/Rampur | 16.4.03 | SK/ YDGC | Cropland | 896 | M29 |
| *B. bassiana* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Cropland | 897 | B1 |
| *M. anisopliae* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Cropland | 898 | M30 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *M. anisopliae* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Cropland | 899 | M31 |
| *M. anisopliae* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Cropland | 900 | M32 |
| *B. bassiana* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Grassland | 901 | B2 |
| *B. bassiana* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Grassland | 902 | B3 |
| *B. bassiana* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Grassland | 903 | B4 |
| *B. bassiana* | GBM | Rishing Patan | 16.4.03 | SK/ YDGC | Grassland | 904 | B5 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 905 | M33 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 906 | M34 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 907 | M35 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 908 | M36 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 909 | M37 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Grassland | 910 | M38 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Grassland | 911 | M39 |
| *M. anisopliae* | GBM | Pang/Parbat | 16.4.03 | SK/ YDGC | Cropland | 912 | M40 |
| *M. anisopliae* | GBM | Pang/Parbat | 23.4.03 | SK/ YDGC | Cropland | 913 | M41 |
| *M. anisopliae* | GBM | Pang/Parbat | 23.4.03 | SK/ YDGC | Grassland | 914 | M42 |
| *M. anisopliae* | GBM | Pang/Parbat | 28.4.03 | SK/ YDGC | Grassland | 915 | M43 |
| *M. anisopliae* | GBM | Pang/Parbat | 28.4.03 | SK/ YDGC | Grassland | 916 | M44 |
| *M. anisopliae* | GBM | Pang/Parbat | 23.4.03 | SK/ YDGC | Grassland | 917 | M45 |
| *B. bassiana* | GBM | Pang/Parbat | 15.4.03 | SK/ YDGC | Cropland | 918 | B6 |
| *M. anisopliae* | SM/soil | Pang/Parbat | 10.4.03 | SK/ YDGC | Grassland | 919 | M46 |
| *M. anisopliae* | SM/soil | Pang/Parbat | 7.5.03 | SK/ YDGC | Grassland | 920 | M47 |
| *M. anisopliae* | White grubs | Pang/Parbat | 12.6.03 | Y.D. GC | Cropland | | M48 |
| *M. anisopliae* | White grubs | Pang/Parbat | 17.6.03 | Y.D. GC | Cropland | | M49 |
| *M. anisopliae* | White grubs | Pang/Parbat | 9.10.03 | Y.D. GC | Cropland | | M50 |
| *M. anisopliae* | GBM | Pang/Parbat | 13.7.03 | Y.D. GC | Cropland | | M51 |
| *M. anisopliae* | GBM | Pang/Parbat | 13.7.03 | Y.D. GC | Cropland | | M52 |
| *M. anisopliae* | GBM | Chitwan | 21.8.03 | Y.D. GC | Cropland | | M53 |
| *M. anisopliae* | White grubs | Chitwan | 21.10.03 | Y.D. GC | Cropland | | M54 |
| *M. anisopliae* | White grubs | Chitwan | 25.10.03 | Y.D. GC | Cropland | | M55 |
| *B. bassiana* | White grubs | Chitwan | 17.11.03 | Y.D. GC | Cropland | | B7 |
| *B. bassiana* | White grubs | Chitwan | 17.11.03 | Y.D. GC | Cropland | | B8 |
| *M. anisopliae* | White grubs | Chitwan | 25.11.03 | Y.D. GC | Cropland | | M56 |
| *M. anisopliae* | White grubs | Chitwan | 27.11.03 | Y.D. GC | Cropland | | M57 |
| *M. anisopliae* | White grubs | Gaindakot | 15.1.04 | Y.D. GC | Cropland | | M58 |
| *M. anisopliae* | White grubs | Gaindakot | 13.2.04 | Y.D. GC | Cropland | | M59 |
| *M. anisopliae* | White grubs | Chitwan | 4.3.04 | Y.D. GC | Cropland | | M60 |
| *M. anisopliae* | White grubs | Tanahun | 15.3.04 | Y.D. GC | Cropland | | M61 |
| *M. anisopliae* | White grubs | Chitwan | 11.4.04 | Y.D. GC | Cropland | | M62 |
| *M. anisopliae* | White grubs | Chitwan | 25.4.04 | Y.D. GC | Cropland | | M63 |
| *M. anisopliae* | White grubs | Tanahun | 22.5.04 | Y.D. GC | Cropland | | M64 |
| *M. anisopliae* | White grubs | Tanahun | 29.5.04 | Y.D. GC | Cropland | | M65 |
| *M. anisopliae* | White grubs | Chitwan | 7.7.04 | Y.D. GC | Cropland | | M66 |
| *M. anisopliae* | White grubs | Gaindakot | 15.7.04 | Y.D. GC | Cropland | | M67 |
| *M. anisopliae* | White grubs | Tanahun | 6.8.04 | Y.D. GC | Cropland | | M68 |
| *M. anisopliae* | White grubs | Chitwan | 15.8.04 | Y.D. GC | Cropland | | M69 |
| *M. anisopliae* | White grubs | Chitwan | 21.8.04 | Y.D. GC | Cropland | | M70 |

During this study *M. anisopliae* and *B. bassiana* were recovered for the first time in Nepal (Table 4.1). Further, it was demonstrated that the natural infection of white grubs is low and that *M. anisopliae* is wide-spread in soils in Nepal. Insect pathogenic fungi could be isolated from white grubs and soils using GBM as well as selective medium. Fungus inoculums were successfully produced on selective medium under laboratory conditions.

### 4.4.2 Time mortality bioassays with white grubs larvae

The time mortality study (Table 4. 2) indicated that 8 isolates gave over 80% infected grubs, 65 isolates gave 50-60% infected grubs and rest of the isolates had a low pathogenicity (Figure 4.1). Based on infection percentage, the fungus strains M1, M6, M18, M48, and M50 were found aggressive as compared to the rest of the strains. The LT50 varied between 2-9 weeks, 12 isolates were highly virulent with an LT50 of 2-4 weeks, 34 isolates had a moderate virulence with an LT 50 of 5-6 weeks and 22 isolates had a low virulence. It is interesting to note that isolates M1 and M6 appeared more virulent based on their ability to kill the larvae reasonably shorter period of time. These two isolates were selected as candidates for the biological control of white grubs. The white grub species used in both the experiments were later identified as *Maladera affinis* Blanchard.

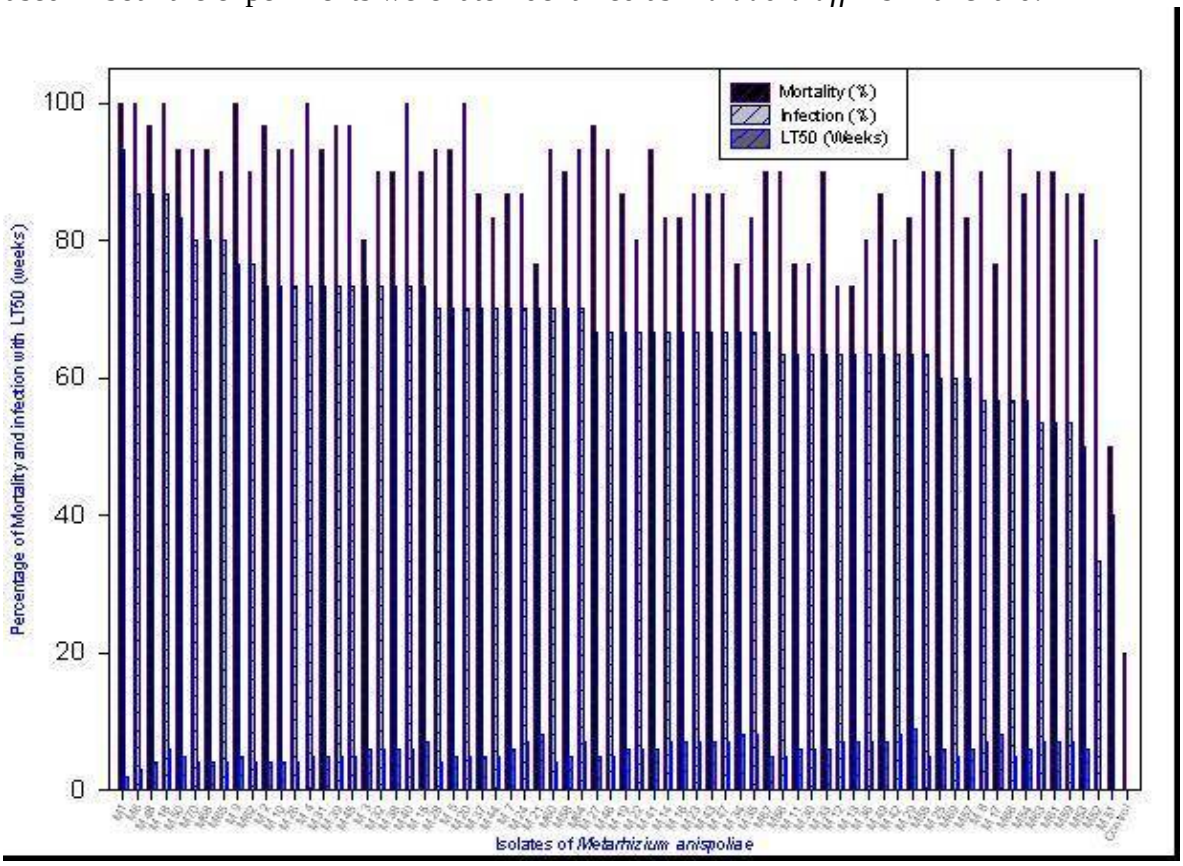


Figure 4.1 Screening of *Metarhizium anisopliae* isolates for infection to white grubs during 2003/04 in IAAS, Rampur, Chitwan, Nepal

From the seventy different fungus isolates tested the five most virulent ones were selected for dose-mortality studies (Table 4.2). This study convincingly showed that isolate M1 resulted significantly higher mortality (100%) virulence (93.3%) ($p< 0.001$) over the tested isolates. Two isolates namely M51 and M52 were found very poor in terms of their efficacy for the infection despite of the result of their better mortality. Interestingly M52 resulted higher mortality (80%) however, caused only 33.3% infection to the white grubs.

Table 4.2 Ranking of fungus isolates with respect to infection percentage ($10^7$spores/ml).

| Isolates (ranked order) | Mortality (%) | Infection (%) | LT50 (Weeks) | Isolates (ranked order) | Mortality (%) | Infection (%) | LT50 (Weeks) |
|---|---|---|---|---|---|---|---|
| M1 | 100.00 | 93.33 | 2 | M 37 | 86.67 | 70.00 | 5 |
| M6 | 100.00 | 86.67 | 3 | M 44 | 83.33 | 70.00 | 5 |
| M 48 | 96.67 | 86.67 | 4 | M 7 | 86.67 | 70.00 | 6 |
| M 18 | 100.00 | 86.67 | 6 | M 24 | 86.67 | 70.00 | 7 |
| M 50 | 93.33 | 83.33 | 5 | M 21 | 76.67 | 70.00 | 8 |
| M70 | 93.34 | 80.00 | 4 | M60 | 93.34 | 70.00 | 4 |
| M68 | 93.34 | 80.00 | 4 | M58 | 90.00 | 70.00 | 5 |
| M65 | 90.00 | 80.00 | 4 | M64 | 93.34 | 70.00 | 7 |
| M 9 | 100.00 | 76.67 | 5 | M 27 | 96.67 | 66.67 | 5 |
| M62 | 90.00 | 76.67 | 4 | M 46 | 93.33 | 66.67 | 5 |
| M 2 | 96.67 | 73.33 | 4 | M 19 | 86.67 | 66.67 | 6 |
| M 10 | 93.33 | 73.33 | 4 | M 22 | 80.00 | 66.67 | 6 |
| M 26 | 93.33 | 73.33 | 4 | M 41 | 93.33 | 66.67 | 6 |
| M 4 | 100.00 | 73.33 | 5 | M 14 | 83.33 | 66.67 | 7 |
| M 31 | 93.33 | 73.33 | 5 | M 16 | 83.33 | 66.67 | 7 |
| M 39 | 96.67 | 73.33 | 5 | M 23 | 86.67 | 66.67 | 7 |
| M 45 | 96.67 | 73.33 | 5 | M 43 | 86.67 | 66.67 | 7 |
| M 3 | 80.00 | 73.33 | 6 | M 47 | 86.67 | 66.67 | 7 |
| M 32 | 90.00 | 73.33 | 6 | M 34 | 76.67 | 66.67 | 8 |
| M 38 | 90.00 | 73.33 | 6 | M 35 | 83.33 | 66.67 | 8 |
| M 40 | 100.00 | 73.33 | 6 | M67 | 90.00 | 66.67 | 5 |
| M 15 | 90.00 | 73.33 | 7 | M56 | 90.00 | 63.34 | 5 |
| M 28 | 93.33 | 70.00 | 4 | M 11 | 76.67 | 63.33 | 6 |
| M 5 | 93.33 | 70.00 | 5 | M 30 | 76.67 | 63.33 | 6 |
| M 20 | 100.00 | 70.00 | 5 | M 33 | 90.00 | 63.33 | 6 |
| M 12 | 73.33 | 63.33 | 7 | M 25 | 90.00 | 60.00 | 6 |
| M 13 | 73.33 | 63.33 | 7 | M69 | 93.34 | 60.00 | 5 |
| M 36 | 80.00 | 63.33 | 7 | M57 | 83.34 | 60.00 | 6 |
| M 49 | 86.67 | 63.33 | 7 | M 8 | 90.00 | 56.67 | 7 |
| M 42 | 80.00 | 63.33 | 8 | M 17 | 76.67 | 56.67 | 8 |
| M 29 | 83.33 | 63.33 | 9 | M66 | 93.34 | 56.67 | 5 |
| M55 | 90.00 | 63.33 | 5 | M54 | 86.67 | 56.66 | 6 |
| M63 | 90.00 | 53.34 | 7 | M53 | 86.67 | 50.00 | 6 |
| M61 | 90.00 | 53.34 | 7 | M52 | 80.00 | 33.33 | 0 |
| M59 | 86.67 | 53.34 | 7 | M 51 | 50.00 | 40.00 | 0 |
| | | | | Control | 20.00 | 0.00 | 0 |

## 4.5 DISCUSSION

The bioassay experiment convincingly showed that some of the strains isolated from the white grubs are found to be more virulent as compared to soil isolates. Hence, further experiments are suggested with such isolates while mass production is carried out towards the progressive start of mycopesticides based programme. The bioassay method is found to be one of the useful approaches of screening of the fungus isolates. The soil fungus is regenerative in the natural environment and resistance build up by the insect pest is less likely. In addition, most of the solely chemical based technologies are likely to be worth for a few years before the insect builds resistance. In this sense, microbial control will be more sustainable approach for the increased farm production. The population of these agents

should be encouraged in the endemic localities of the white grubs either by inoculative or inundative release after mass production.

## 4.6 CONCLUSIONS

The development of commercial products based on entomopathogenic fungi for the use in integrated pest management programs needs several steps. Fungal species and isolates must first be obtained from diseased insects or from the environment, and identified. Techniques for culturing and preservation are subsequently the essential steps. Pathogenicity of the isolates needs to be screened in tired experiments and at the same time it is important to know the tested host and its biology. The most promising candidates are evaluated both in captivity and for field conditions. Handling of the fungus material between production and storage to its ultimate use in the field is very crucial to maintain the viability. Most of the mycopesticides imported from India are carelessly stored in high temperatures in the general agro-vet shops, where there are high chances to loose viability. Therefore, the viable and virulent indigenous materials should be applied into multilocational trail since there is wider variation in the species distribution of white grubs in Nepal. In order to achieve the effective reduction of the white grubs damages, identification of the damaging species is necessary. At the same time, production, application and assessment of the fungus efficacy should be carried out by trained technicians.

This kind of work in Nepal is at the very rudimentary stage despite of its earlier necessity. This work may be geared up with the involvement of the appropriate organizations which have the necessary capacity and the technical expertise. Concentrated efforts are necessary for the successful implementation of the biocontrol activities based on mycopesticides. Working with mycopesticides in Nepal is very favorable since the government and donor organizations have given due priority to this aspect.

The agricultural systems in Nepal are basically run by the involvement of different organizations, where research works are being carried out by Nepal Agricultural Research Council (NARC), Institute of Agriculture and Animal Sciences (IAAS) of Tribhuvan University (TU), RONAST (Royal Nepal Academy of Science and Technology) and by some of the International Organisations (INGOs) and Non-Governmental Organisations (NGOs).) The generated technologies are disseminated by the involvement of governmental (GOs) through the Department of Agriculture (DOA) and various non-governmental organizations (NGOs) at their various capacities. No private companies are involved yet in research and production of BCAs.
There is urgent need of viable collaboration among these partner organizations for the effective promotion of the mycopesticide based activities in Nepal. In the initial period, IAAS may deliver the knowledge regarding microbial control works to the private organizations and support them technically for the method of production and commercialization. Additional research may be carried out by NARC and RONAST.

An outline of the major phases of research and major counterparts towards the microbial control of white grubs in Nepal is proposed in Figure 4.1.

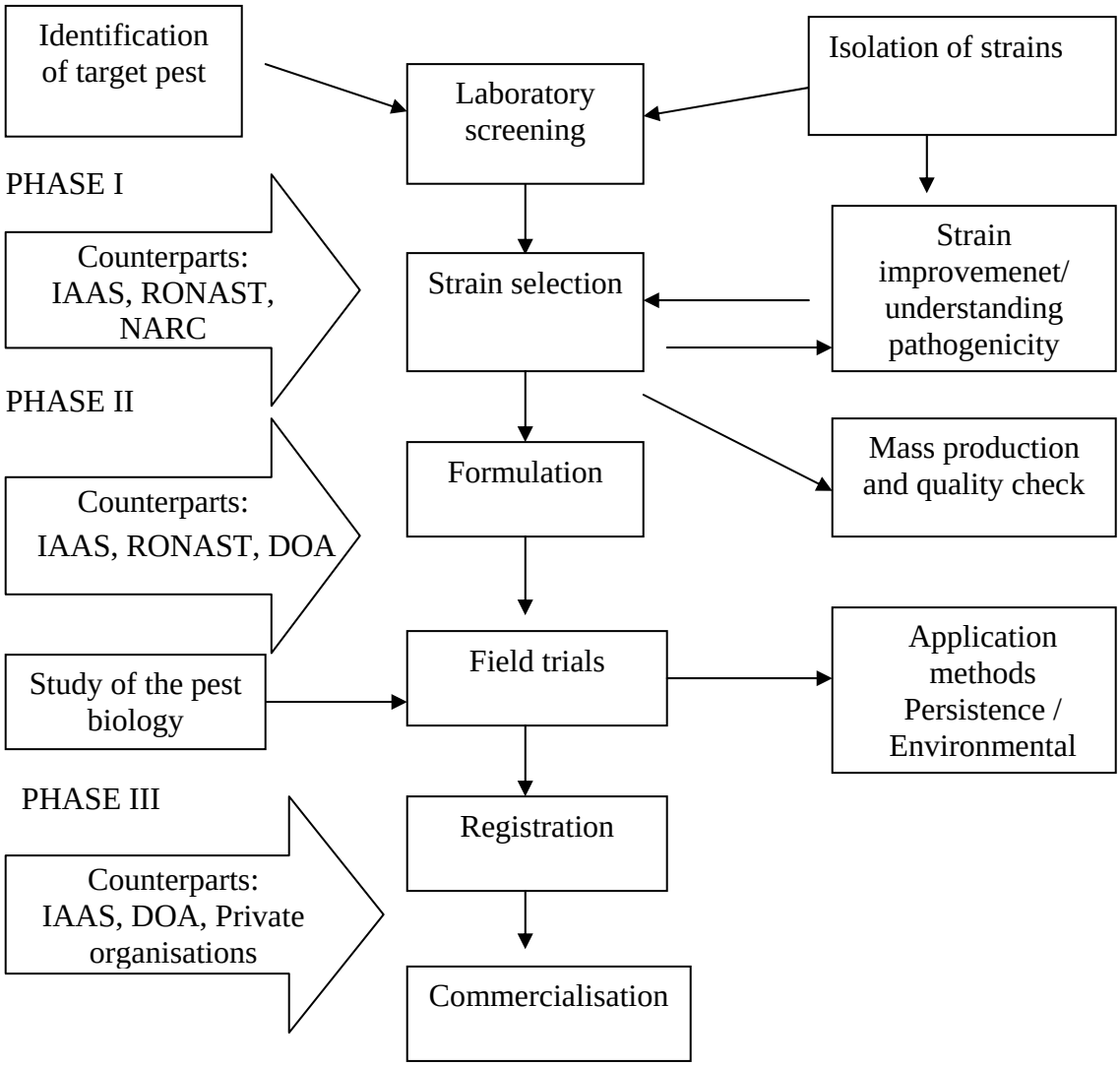


Figure 4.2 Suggested strategies for the development of fungal based mycoinsecticides in Nepal

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Appendix 4.1 Calculation of fungal conidia of *Metarhizium anisopliae* to get required concentration in a bioassay experiment in IAAS, Rampur, Chitwan in 2003/04. Given the area of the smallest unit of Haemocytometer = 0.0025 $mm^2$ and height = 0.1000 mm. The volume of water in the smallest unit = 0.0025 x 0.100 $mm^3$ = 0.00025 $mm^3$

| Isolates | Conc. of big square chamber | Av. count of conidia on big square | Av. conidia in smaller unit of haemocytometer (n) | Conc. of conidia in original stock susp. (spores/ml) n x conversion factor | Stock susp. (ml) | Water added (ml) |
|---|---|---|---|---|---|---|
| M1 | 88 + 118 98+ 108 | 412/4 = 103 | 103/16 = 6.4 | 6.4375 x 4 x $10^6$ = 2.575x $10^7$ | 55.50 | 44.50 |
| M2 | 80 + 98 + 58 + 120 | 356/4 = 89 | 89 /16 = 5.5 | 5.5625 x 4 x $10^6$ = 2.225 x $10^7$ | 45.20 | 54.80 |
| M3 | 60 + 78 + 91 + 99 | 328/4 = 82 | 82 /16 = 5.1 | 5.125x 4 x $10^6$ = 2.0500 x $10^7$ | 65.13 | 34.87 |
| M4 | 78 + 105 + 87 + 78 | 348/4 = 87 | 87 /16 = 5.4 | 5.4375 x 4 x $10^6$ = 2.175 x $10^7$ | 58.20 | 41.80 |
| M5 | 88 + 65 + 89 + 70 | 312/4 = 78 | 78 /16 = 4.8 | 4.875 x 4 x $10^6$ = 1.950 x $10^7$ | 78.12 | 21.88 |
| M6 | 62 + 69 + 81 + 75 | 287/4 = 71 | 71 /16 = 4.4 | 4.437 x 4 x $10^6$ = 1.774 x $10^7$ | 45.69 | 54.31 |
| M7 | 93 + 61 + 97 + 104 | 355/4 = 88 | 88 /16 = 5.5 | 5.500 x 4 x $10^6$ = 2.200 x $10^7$ | 71.03 | 28.97 |
| M8 | 45 + 77 + 91 + 66 | 279/4 = 69 | 69 /16 = 4.3 | 4.312x 4 x $10^6$ = 1.724 x $10^7$ | 56.07 | 43.93 |
| M9 | 32 + 69 + 102 +55 | 258/4 = 64.5 | 64.5 /16 = 4.0 | 4.031x 4 x $10^6$ = 1.612 x $10^7$ | 54.30 | 45.70 |
| M10 | 67 +29 + 84 +45 | 225/4 = 56.2 | 56.25 /16 = 3.5 | 3.515 x 4 x $10^6$ = 1.406 x $10^7$ | 43.05 | 56.95 |
| M11 | 49 +43 + 55 +101 | 248/4 = 56.2 | 62 /16 = 3.8 | 3.875 x 4 x $10^6$ = 1.550 x $10^7$ | 66.02 | 33.98 |
| M12 | 80 + 65 + 89 + 78 | 312/4 = 78 | 78 /16 = 4.8 | 4.875 x 4 x $10^6$ = 1.950 x $10^7$ | 45.46 | 54.54 |
| M13 | 93 + 76+ 82 + 104 | 355/4 = 88 | 88 /16 = 5.50 | 5.500 x 4 x $10^6$ = 2.200 x $10^7$ | 53.08 | 46.92 |
| M14 | 47+55 + 79 +108 | 289/4 = 72.2 | 72.25 /16 = 4.5 | 4.515 x 4 x $10^6$ = 1.806 x $10^7$ | 78.02 | 21.98 |
| M15 | 51 +56 + 23+105 | 236/4 = 59.0 | 59.0 /16 = 3.6 | 3.687 x 4 x $10^6$ = 1.475 x $10^7$ | 56.45 | 43.55 |
| M16 | 40 + 85 + 89 + 98 | 312/4 = 78 | 78 /16 = 4.87 | 4.875 x 4 x $10^6$ = 1.950 x $10^7$ | 81.23 | 18.77 |
| M17 | 39 +53 + 50 +96 | 248/4 = 56.2 | 62 /16 = 3.87 | 3.875 x 4 x $10^6$ = 1.550 x $10^7$ | 52.31 | 47.69 |
| M18 | 87 +105 + 45+52 | 299/4 = 74.7 | 74.75 /16 = 4.6 | 4.671 x 4 x $10^6$ = 1.868 x $10^7$ | 85.21 | 14.79 |
| M19 | 21 +115 + 46+91 | 273/4 = 68.2 | 68.25 /16 = 4.2 | 4.265 x 4 x $10^6$ = 1.706 x $10^7$ | 79.23 | 20.77 |
| M20 | 41 +43 + 33+86 | 203/4 = 59.0 | 50.75 /16 = 3.1 | 3.171 x 4 x $10^6$ = 1.268 x $10^7$ | 77.33 | 22.67 |
| M21 | 55 + 39+ 76 + 94 | 294/4 = 66 | 66 /16 = 4.12 | 4.125 x 4 x $10^6$ = 1.650 x $10^7$ | 69.65 | 30.35 |
| M22 | 82 +49 + 57 + 87 | 275/4 = 68.7 | 68.75 /16 = 4.2 | 4.296 x 4 x $10^6$ = 1.718 x $10^7$ | 56.48 | 43.52 |
| M23 | 68 +47+ 85+108 | 308/4 = 77 | 77 /16 = 4.8 | 4.812 x 4 x $10^6$ = 1.925 x $10^7$ | 69.45 | 30.55 |
| M24 | 56 +87 +31+55 | 229/4 = 57.2 | 57.25/16= 3.5 | 3.578 x 4 x $10^6$ = | 78.23 | 21.77 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | $1.431 \times 10^7$ | | |
| M25 | 27+75 + 88 +99 | 289/4 = 72.2 | 72.25 /16 = 4.5 | $4.515 \times 4 \times 10^6 = 1.806 \times 10^7$ | 56.55 | 43.45 |
| M26 | 26+82+ 107+54 | 269/4 = 67.2 | 67.25 /16 = 4.2 | $4.203 \times 4 \times 10^6 = 1.681 \times 10^7$ | 72.23 | 27.77 |
| M27 | 51+73 + 68 +95 | 287/4 = 71.7 | 71.75 /16 = 4.4 | $4.484 \times 4 \times 10^6 = 1.793 \times 10^7$ | 81.23 | 18.77 |
| M28 | 39+41 + 26 +54 | 160/4 = 40.0 | 40 /16 = 2.5 | $2.500 \times 4 \times 10^6 = 1.000 \times 10^7$ | 87.23 | 12.77 |
| M29 | 31+44 + 46 +79 | 200/4 = 50.0 | 50 /16 = 3.1 | $3.125 \times 4 \times 10^6 = 1.2500 \times 10^7$ | 67.64 | 32.36 |
| M30 | 27+54 +89 +54 | 224/4 = 56 | 56 /16 = 3.5 | $3.500 \times 4 \times 10^6 = 1.400 \times 10^7$ | 83.50 | 18.50 |
| M31 | 74+49 + 66 +71 | 260/4 = 65 | 65 /16 = 4.0 | $4.062 \times 4 \times 10^6 = 1.625 \times 10^7$ | 84.00 | 16.00 |
| M32 | 37+45 +51 +78 | 211/4 = 52.7 | 52.75 /16 = 3.2 | $3.296 \times 4 \times 10^6 = 1.318 \times 10^7$ | 65.46 | 34.54 |
| M33 | 29+35 + 67 +76 | 207/4 = 51.7 | 51.75 /16 = 3.2 | $3.245 \times 4 \times 10^6 = 1.293 \times 10^7$ | 67.56 | 32.44 |
| M34 | 40+51 + 49 +47 | 187/4 = 46.7 | 46.75 /16 = 2.9 | $2.921 \times 4 \times 10^6 = 1.168 \times 10^7$ | 56.38 | 43.62 |
| M35 | 69+71 + 36 +64 | 240/4 = 60 | 60 /16 = 3.7 | $3.75 \times 4 \times 10^6 = 1.500 \times 10^7$ | 70.65 | 29.35 |
| M36 | 75+27 + 68 +67 | 237/4 = 59.2 | 59.25 /16 = 3.7 | $3.703 \times 4 \times 10^6 = 1.481 \times 10^7$ | 79.56 | 20.44 |
| M37 | 44+43 + 71 +91 | 249/4 = 62.2 | 62.25 /16 = 3.8 | $3.890 \times 4 \times 10^6 = 1.556 \times 10^7$ | 75.21 | 24.79 |
| M38 | 56+59 +38 +64 | 217/4 = 54.2 | 54.25 /16 = 3.3 | $3.390 \times 4 \times 10^6 = 1.356 \times 10^7$ | 82.65 | 17.35 |
| M39 | 77+37 + 55 +46 | 215/4 = 53.7 | 53.75 /16 = 3.3 | $3.359 \times 4 \times 10^6 = 1.343 \times 10^7$ | 59.56 | 40.44 |
| M40 | 64+53 +72 +101 | 290/4 = 72.5 | 72.50 /16 = 4.5 | $4.531 \times 4 \times 10^6 = 1.812 \times 10^7$ | 67.84 | 32.16 |
| M41 | 74+59 + 66 +61 | 260/4 = 65 | 65 /16 = 4.0 | $4.062 \times 4 \times 10^6 = 1.625 \times 10^7$ | 76.58 | 23.42 |
| M42 | 35+39 + 84 +54 | 212/4 = 53 | 53 /16 = 3.3 | $3.312 \times 4 \times 10^6 = 1.325 \times 10^7$ | 67.56 | 32.44 |
| M43 | 64+39 + 56 +61 | 220/4 = 55 | 55 /16 = 3.4 | $3.437 \times 4 \times 10^6 = 1.375 \times 10^7$ | 59.65 | 40.35 |
| M44 | 55+48 + 54 +46 | 203/4 = 50.7 | 50.57 /16 = 3.1 | $3.171 \times 4 \times 10^6 = 1.268 \times 10^7$ | 78.56 | 21.44 |
| M45 | 60+31 + 46+55 | 192/4 = 48 | 48 /16 = 3.0 | $3.000 \times 4 \times 10^6 = 1.200 \times 10^7$ | 79.21 | 20.79 |
| M46 | 69+42 +58 +89 | 258/4 = 64.5 | 64.5 /16 = 4.0 | $4.031 \times 4 \times 10^6 = 1.612 \times 10^7$ | 81.23 | 18.77 |
| M47 | 74+59 +77 +51 | 261/4 = 65.2 | 65.25 /16 = 4.0 | $4.078 \times 4 \times 10^6 = 1.631 \times 10^7$ | 67.13 | 32.87 |
| M48 | 30+49 + 94 +44 | 217/4 = 54.2 | 54.25 /16 = 3.3 | $3.390 \times 4 \times 10^6 = 1.356 \times 10^7$ | 79.46 | 20.54 |
| M49 | 50+71 + 42+47 | 210/4 = 52.5 | 52.5 /16 = 3.2 | $3.281 \times 4 \times 10^6 = 1.312 \times 10^7$ | 67.45 | 32.55 |
| M50 | 63+69 +75 +41 | 248/4 = 62 | 62 /16 = 3.8 | $3.875 \times 4 \times 10^6 = 1.550 \times 10^7$ | 91.23 | 8.77 |
| M51 | 74+59 +77 +51 | 261/4 = 65.2 | 65.25 /16 = 4. | $4.078 \times 4 \times 10^6 = 1.631 \times 10^7$ | 67.13 | 32.87 |
| M52 | 24+36 + 42 +41 | 143/4 = 37.7 | 35.75 /16 = 2.2 | $2.234 \times 4 \times 10^6 = 0.893 \times 10^7$ | 76.45 | 23.55 |

| | | | | | | |
|---|---|---|---|---|---|---|
| M53 | 34+65 + 39 +81 | 219/4 = 54.7 | 54.75 /16 = 3.4 | 3.421 x 4 x $10^6$ = 1.368 x $10^7$ | 89.20 | 10.80 |
| M54 | 51+39 +56 +51 | 197/4 = 49.2 | 49.25 /16 = 3.0 | 3.078 x 4 x $10^6$ = 1.231 x $10^7$ | 63.45 | 36.55 |
| M55 | 50+29 +84 +104 | 267/4 = 66.7 | 66.75 /16 = 4.1 | 4.171 x 4 x $10^6$ = 1.668 x $10^7$ | 84.56 | 15.44 |
| M56 | 34+66 +72 +41 | 213/4 = 53.2 | 53.25 /16 = 3.3 | 3.328 x 4 x $10^6$ = 1.331 x $10^7$ | 60.31 | 39.69 |
| M57 | 48+109 +71 +41 | 269/4 = 67.2 | 67.25 /16 = 4.2 | 4.203 x 4 x $10^6$ = 1.681 x $10^7$ | 56.52 | 43.48 |
| M58 | 110+69 +78 +50 | 307/4 = 76.7 | 76.75 /16 = 4.7 | 4.796 x 4 x $10^6$ = 1.981 x $10^7$ | 70.56 | 29.44 |
| M59 | 68+34 +98+61 | 261/4 = 65.2 | 65.25 /16 = 4.0 | 4.078 x 4 x $10^6$ = 1.631 x $10^7$ | 45.67 | 54.33 |
| M60 | 40+58 +64+90 | 252/4 = 63.0 | 63.00 /16 = 3.9 | 3.937 x 4 x $10^6$ = 1.575 x $10^7$ | 52.39 | 47.61 |
| M61 | 60+58 + 84+77 | 279/4 = 69.7 | 69.75 /16 = 4.3 | 4.359 x 4 x $10^6$ = 1.743 x $10^7$ | 67.65 | 32.35 |
| M62 | 34+44 +50 +72 | 200/4 = 50.0 | 50.00 /16 = 3.1 | 3.125 x 4 x $10^6$ = 1.250 x $10^7$ | 57.12 | 42.88 |
| M63 | 70+34 +54 +75 | 233/4 = 58.2 | 58.25 /16 = 3.6 | 3.640 x 4 x $10^6$ = 1.456 x $10^7$ | 54.61 | 45.39 |
| M64 | 78+49 +35 +64 | 226/4 = 56.5 | 56.50 /16 = 3.5 | 3.531 x 4 x $10^6$ = 1.412 x $10^7$ | 55.45 | 44.55 |
| M65 | 38+69 +89 +54 | 250/4 = 62.5 | 62.50 /16 = 3.9 | 3.906 x 4 x $10^6$ = 1.562 x $10^7$ | 79.46 | 20.54 |
| M66 | 63+58 + 56+75 | 252/4 = 63.0 | 63.00 /16 = 3.9 | 3.937 x 4 x $10^6$ = 1.575 x $10^7$ | 68.34 | 31.66 |
| M67 | 41+66 +84+53 | 244/4 = 61.0 | 61.00 /16 = 3.8 | 3.812 x 4 x $10^6$ = 1.525 x $10^7$ | 57.56 | 42.44 |
| M68 | 111+75 +54+81 | 321/4 = 80.2 | 80.25 /16 = 5.0 | 5.016 x 4 x $10^6$ = 2.006 x $10^7$ | 47.65 | 52.35 |
| M69 | 43+68 + 46+82 | 239/4 = 59.7 | 59.75 /16 = 3.7 | 3.734 x 4 x $10^6$ = 1.493 x $10^7$ | 56.32 | 43.68 |
| M70 | 88+65 +109+65 | 327/4 =81.7 | 81.75 /16 = 5.1 | 5.109 x 4 x $10^6$ = 2.043 x $10^7$ | 58.65 | 41.35 |

Appendix 4.2 Monthly average temperatures and relative humidity during the bioassay experiment in the laboratory of IAAS, Rampur, Chitwan in 2004.

| Month | Room temperature ($^o$C) | | Relative humidity (%) | |
|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum |
| January | 19.5 | 16.2 | 75.4 | 67.1 |
| February | 22.3 | 20.1 | 70.1 | 61.2 |
| March | 24.6 | 20.4 | 82.0 | 65.5 |
| April | 25.0 | 21.1 | 70.8 | 54.9 |
| May | 24.2 | 20.3 | 63.1 | 53.8 |
| June | 24.9 | 21.6 | 75.8 | 71.2 |
| July | 23.6 | 21.3 | 81.2 | 74.3 |
| August | 22.9 | 21.1 | 82.7 | 75.3 |
| September | 23.5 | 20.9 | 86.4 | 79.1 |
| October | 23.4 | 19.6 | 85.3 | 78.3 |
| November | 22.3 | 18.1 | 86.8 | 76.4 |
| December | 23.6 | 18.3 | 76.2 | 70.2 |

# Chapter 5

## Pathogenicity of strains of the entomopathogenic fungus, *Metarhizium anisopliae* against larvae of the tropical species, *Maladera affinis* Blanchard, through bioassay experiment



Part of these results has been published in the Tribhuvan University Journal (Yubak Dhoj G. C. *et al.* 2004).

# Chapter 5

# Pathogenicity of strains of the entomopathogenic fungus, *Metarhizium anisopliae* against larvae of the tropical species, *Maladera affinis* Blanchard, through bioassay experiment

## 5.1 SUMMARY

In the initial screening experiment, a total of seventy different isolates of insect pathogenic fungus (*Metarhizium anisopliae)*, were assessed at the Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan, Nepal, in 2003/04. Among them, only five isolates (three originated from natural insect hosts and two from soil sources) were promoted in the second tired experiment. In order to determine the most virulent isolates, further screening was performed in a bioassay experiment and effective dosages in terms of LT 50 and LC 50 were depicted from such virulent isolates under laboratory conditions. The isolate, *M. anisopliae* M1 was found one of the most virulent strains as compared to the tested isolates ($p<0.010$). Two different types of inocula such as conidia and blastospores were assessed. In one set of experiment, only the conidial concentration of the fungus at four different dosages ($10^9$, $10^5$, $10^2$ spores/ml and dipping in water) were evaluated against third instar larvae of white grubs. The onset of cumulative mortality and mycosis suggests grubs were moderately to highly susceptible to the fungi with all the dosages, however, higher dosages are more effective compared to lower dosages ($p<0.010$). In another set of experiments, comparative study of pathogenicity with two different types of inocula, conidiospores and blastospores, against three different instars of white grubs showed that second instar larvae are more affected ($p< 0.010$) than first and third instars. The study on different instars' of the insect would be helpful in targeting the vulnerable instars in conjunction with ample field monitoring. Dead larvae were subsequently placed on damp soil to induce fungus growth from infected grubs with subsequent production of conidia. Different aspects of virulence and pathogenicity have been discussed in relation to white grub control. Based on the virulence M1 isolates of the fungus *M. anisopliae* was promoted for mass production for field application.

## 5.2 INTRODUCTION

Pathogencity and virulence of a microbial insecticide are a function of many interacting factors and are relative to a specific set of conditions which exist between the microbial agent, a particular host and the environment. Virulence refers to the intensity of the disease caused by a pathogen, whereas pathogenicity refers to an organism's ability to cause disease. A fungal species which is pathogenic on a wide range of hosts may be more likely to persist in an environment because of the availability of alternate hosts. With a greater number of susceptible hosts, there may be a greater reservoir of inoculum available to produce an epizootic (Tanada, 1963). Virulence may be measured in the assessment experiments by exposing to known number of hosts with a known number of pathogens and observing them as dead number of treated host over certain period of time (Meynell and Meynell, 1965). The lethal concentration (LC) which produces mortality in 50% of the test hosts ($LC_{50}$) is generally used to compare two pathogens or the response of two hosts. The host range of *M. anisopliae* is wide exceeding 200 species of seven orders of the insects (Roberts and Yendol, 1971). The pathogenicity for a host species however varies with strains or isolates (Aizawa, 1987), pathogen

population, dispersal and survival in the host's environment, and inoculum density and spatial distribution (Tanada and Fuxa, 1987). Therefore, the selection of effective strains of entomopathogens is essential for the development of microbial insecticides.

## 5.3 MATERIAL AND METHODS

### 5.3.1 Maintenance of fungus cultures

The strains of *M. anisopliae* used in this experiment were isolated from infected larvae. Five of the most aggressive fungal strains were obtained from the first tired experiment i.e. preliminary screening experiment. The isolates were maintained on Saboraud Dextrose Agar (SDA) supplemented with antibiotics and kept at the insect pathology laboratory of IAAS, Rampur, Nepal, within the framework of a Nepal-Swiss biocontrol project. Each of the isolates was put into regular host passages using the natural hosts. SDA consisted of 100 g dextrose, 50 g peptone, 90 g agar-agar, 0.25 g of cyclohexamide and tetracycline each and 3 g streptomycin to which 1000 ml of distilled water was added. The medium was transferred into sterilized petriplates and test tubes. After inoculation they were incubated at 27±2 $^{o}$C 80±5 % RH to induce growth and sporulation of the fungus.

### 5.3.2 Maintenance of host insects

The grubs were collected from damaged crop fields at different sites of Chitwan (230 m asl) and Nawalparasi (175 m asl). The fields were dug beneath the crop root level and the grubs were picked up and placed individually in poly pots of 4.5 cm diameter and 6 cm height. In order to avoid stress, they were supplied with sterilized wet soils and slices of potato and transported to the lab with care. The grubs were further grouped according to larval instars according to the experimental purposes. They were put into quarantine at a temperature of 22-23 $^{o}$C for five weeks to reduce or avoid natural infections. The larvae were fed with slices of potato as food. The soil as well as food was changed each week. Naturally infected larvae were discarded and only apparently healthy larvae were used in bioassay experiments. The quarantined larvae of different instars were assessed in bioassay experiments using conidiospores and blastospores in a randomized complete block design (RCBD).

### 5.3.3 Production of fungus inocula for bioassays

Isolates of *M. anisopliae* were maintained on SDA for no longer than two passages. Two different sources of inoculums such as conidiospores as well as blastospores were evaluated in the bioassay experiments. Conidia were produced on the selective medium adapted from Strasser *et al.* (1997) as described in Chapter 4. The conidia were harvested by scrapping off the contents of each Petri dish or test tube with a sterile bacteriological loop. The conidial mass was dispersed in distilled water by using a drop of Tween 80 since they are hydrophobic in nature. From the original concentration, 1 ml stock solution was drawn and the spores counted under the microscope using a Thoma haemocytometer.

Blastospores were produced using the liquid medium described by Blachère *et al.* (1973) with the following composition and preparation: Solution I, with 500 ml tap water, 20 g Corn steep, 2.26 g $KH_2PO_4$, 3.8 g $Na_2HPO_4$ and solution II with 500 ml tap water, 30 g Glucose or Sucrose. The two solutions were autoclaved separately at a

temperature of 120 $^{o}$C during 40 minutes. The two solutions were mixed under sterile conditions and poured in autoclaved Erlenmeyer flasks of the preferred size with 100 ml liquid medium. After cooling, they were inoculated with a small piece of agar with mycelium and conidia of *M. anisopliae* from a Petri dish of the second generation. The isolate M1 was used for the experiments. The fungi were cultivated on a longitudinal shaker at 100 rpm at 20 $^{o}$C for six days. The growth of *Metarhizium* became visible when white mycelium with green conidia developed at the edge of liquid medium. The resulting spores were counted in a Thoma haemocytometer. For the assays carried out, culture broths were diluted 100 times with sterile water and 0.01 % Tween 80 from their original concentration following the procedures as described in the Appendix 5.1.

### 5.3.4 Pathogenicity test in bioassay experiment

In this study two sets of experiments were conducted using the dipping method (Goettel and Inglis, 1997). In the first set of experiment, conidiospores of the five selected, virulent strains M1, M6, M18, M48 and M50 were assessed with a single dose of $10^7$ spores /ml. All the strains tested in this experiment were promoted (Koch's postulates) from the initial screening study as described in Chapter 4. After selecting the most virulent strains from the initial screening, four different treatments (concentrations) such as $10^9$ spores /ml, $10^5$ spores /ml, $10^2$ spores /ml and control were assessed in randomized complete block design (RCBD). The virulence in both cases was assessed against third instar larvae of *Maladera* sp for 75 days at a temperature of 22-23 $^{o}$C.

In the second experiment a concentration of $10^7$ spores /ml of the most virulent strain of *M. anisopliae*, M1, was assessed against the three larval instars (L1, L2 and L3). The treatments were laid out in a CRBD considering two different factors such as fungus inoculum (conidiospores and blastospore) and insect instar (first, second and third instar). The bioassay in both sets of experiment was performed with a group of 30 white grubs at a temperature of 22-24 $^{o}$C. Bioassays were performed by dipping to first instar, second instar and third instar larvae in blastospore suspensions for five seconds. The grubs were submerged for 30 seconds in 50 ml of conidial concentration with $10^7$ spores/ml. After allowing the excess liquid to drip off, the larvae were placed individually in rearing pots (cylindrical, inner diameter 45 mm, height 60 mm) filled 2/3 rd with sterile soil. Sliced potatoes serving as food were added to the peat. The larvae were checked at weekly intervals for fungal infections. The peat was replaced as necessary. The lid of the rearing pots was perforated to facilitate aeration. One group of white grubs was maintained as control just dipping in distilled water. The parameters collected in this experiment were mortality and mycosis as induced by *M. anisopliae*. Recording was carried out at weekly intervals until the period of 75 days after treatment. The percentage mortality was calculated from the total number of live grubs to the total number of dead grubs. In order to induce the mycosis all the dead grubs were transferred to wet soil. The mycosed grubs were calculated from the total number of cadavers with sporulating fungus to the number of dead grubs. The recorded parameters were analyzed using MSTAT software computer package.

## 5.4 RESULTS

### 5.4.1 Maintenance of host insects

White grubs larvae were sufficiently and routinely maintained into the locally available poly pots with slices of potato and /or carrots as food. The temperature (22-24 $^{o}$C) inside

the rearing room was found conducive for the life processes of white grubs. However, there was failure of the power in certain intervals during rearing which caused fluctuation of the temperature in the rearing room, therefore, considerable number of larvae died due to stresses. Interestingly, not much natural infection with insect pathogenic fungi was recorded in this experiment and it was almost below than 0.40 %. The quarantined and non-infected larvae were used for the bioassay experiment (Table 1).

Table 5.1 Origin of the white grubs larvae used in the bioassay experiments in 2005, Rampur, Chitwan, Nepal.

| SN | Locality of collection (origin of the white grubs) | Number of white grubs quarantined for experiments | | |
|---|---|---|---|---|
| | | Observed | Infected with fungus | % fungus infection |
| 1 | Gunganagar, Chitwan | 325 | 1 | 0.30 |
| 2 | Saradanagar, Chitwan | 325 | 0 | 0.00 |
| 3 | IAAS, Livestock farm, Chitwan | 325 | 1 | 0.30 |
| 4 | IAAS, mango orchard, Chitwan | 325 | 2 | 0.61 |
| 5 | Milanchowk, Gaindakot, Nawalparasi | 200 | 0 | 0.00 |
| 6 | Riverside, Gaindakot, Nawalparasi | 200 | 1 | 0.50 |

After quarantine, only the uninfected grubs were used for the experiments, where a total of 450 grubs were used for the first set of experiment (5 isolates x 3 replications x 30 grubs per replications = 450 grubs) and 270 white grubs for second experiments (3 white grubs instars x 3 replications x 30 grubs per replication).

### 5.4.2 Pathogenicity test in bioassay experiment

Pathogenicity of different strains of *M. anisopliae* with a concentration of $10^7$spores/ ml against third instar larvae of *Maladera affinis* are presented in Table 5.2. The result of the first set of experiments with different isolates has indicated that all the isolates are moderately pathogenic to third instar larvae of white grubs. Isolate M1 of *M. anisopliae* found comparatively pathogenic (67.50 % mortality and 40.83 % mycosis) as compared to other isolates tested.

Table 5.2 Pathogenicity of different strains of *M. anisopliae* against third instar larvae of *M. affinis* at 22-24 $^oC$ at a concentration of 1 x $10^7$ spores/ml.

| Fungus strain | Fungus origin | Mortality (%) | Mycosis (%) |
|---|---|---|---|
| M1 | White grub | 67.5 a | 40.8 a |
| M6 | White grub | 64.2 ab | 38.3 ab |
| M18 | Soil/GBM | 59.2 b | 26.7 b |
| M48 | Soil/GBM | 58.3 b | 29.2 ab |
| M50 | White grub | 59.2 b | 30.8 ab |
| Untreated | Water solution | 58.1 b | 0.00 c |
| LSD (p=0.01) | | 6. 953 | 10.92 |
| SEM | | 2.357 | 2.713 |
| CV% | | 10.81 | 23.14 |

Figures in column followed by same letters are not significantly different at p<0.010 by DMRT

The mycosis of different fungus isolates ranged between 26.7% and 40.83%. This experiment has showed, there is more than 50% larval mortality with all the isolates however, the mycosis has not reached to that extent. The possible reason might be due to unfavorable environment for the host and for fungus germination, virulence of the pathogen and host interaction. In addition to the percent mortality, the fungus induced mortality was considered as one of the parameters for the selection of the strains for further experiment. In general, the mortality of the insect after treatment was more or less corresponding in inducing the mycosis in grubs' cadaver except in the isolate M18. Based on these data, isolate M1 was found to be the most effective one in causing mortality and inducing mycosis among the tested grubs. Therefore, it was selected for further steps of pathogeniciy assessment with different doses of conidiospores against third instars white grubs' larvae (Table 5.3).

Table 5.3 Pathogenicity of different doses of isolate M1 of *M. anisopliae* against third instar larvae of *M. affinis* at 22-24 $^{o}$C after 75 days.

| Fungus strain | Fungus doses | Mortality (%) | Mycosis (%) |
|---|---|---|---|
| M1 | $10^{9}$spores/ml | 79.33a | 52.00a |
| | $10^{5}$spores/ml | 72.67ab | 45.33a |
| | $10^{2}$spores/ml | 70.00b | 35.33b |
| | Control | 24.67c | 0.00c |
| | LSD (p=0.010) | 8.483 | 9.764 |
| | SEM | 2.108 | 2.427 |
| | CV% | 10.81 | 23.14 |

Figures in column followed by same letter are not significantly different at p<0.010 by DMRT

The result presented in Table 5.3 showed that the two higher dosages of fungus caused a higher level of mycosis (52%; p<0.010) as compared to the lowest dose. Experiment with different fungus dosages has showed the differences between the highest and the lowest dosages both for mortality and mycosis to white grub larvae.

The results of the second set of experiment with different forms of inoculum to different instars of white grubs are presented in Table 5.4. This experiment showed no differences for mortality of the grubs due to the use of different type of inocula however it was different for infection. At the same time, conidiospores has caused higher rate of infection to the first instars larvae than that of second and third. The fungus inoculum produced as blastospres has caused higher infection and this parameter is found significant (p<0.050) to the second instar larvae than that of first and third instars.

Table 5.4 Effect of fungus inocula on different instars (L1/L2/L3) of white grubs of *M. affinis* after 75 days.

| Inocula | Mortality (%) of different instars | | | Infection (%) of different instars | | |
|---|---|---|---|---|---|---|
| | 1$^{st}$ | 2$^{nd}$ | 3$^{rd}$ | 1$^{st}$ | 2$^{nd}$ | 3$^{rd}$ |
| Conidiospores ($1x10^{7}$spores/ml) | 73.3 | 70.0 | 56.7 | 60.0 | 40.0 | 40.0 |
| Blastospores ($1x10^{7}$spores/ml) | 86.7 | 86.7 | 73.3 | 70.0 | 83.0 | 56.0 |
| LSD (5%) | | 16.63 | | | 16.63 | |
| SEM | | 3.85 | | | 3.85 | |
| CV% | | 9.00 | | | 11.40 | |

Similarly, blastospores caused significantly higher infections ($p<0.001$) and mortality (82.20 %) than conidia (Table 5.5). The possible reason of higher infection to the larval body with blastospores might be due to the full development of penetration pegs than the conidiospores.

Table 5.5 Effect of fungus inocula on the mortality and infection to white grubs larvae in IAAS, Rampur, Chitwan, Nepal (22-23 $^{o}$C) in 2005.

| Fungal inoculum | Mortality % | Infection % |
|---|---|---|
| Conidiospores ($1x10^7$spores/ml) | 66.70 | 46.70 |
| Blastospores ($1x10^7$spores/ml) | 82.20 | 70.00 |
| LSD (5%) | 9.60 | 9.60 |
| SEM | 2.22 | 2.22 |
| CV% | 9.00 | 11.40 |

## 5.5 DISCUSSION

The onset of higher rate of mortality and infection as induced by some fungus strains might be due to host factors than that of pathogen, however, the reason behind this is not clear, therefore, further studies in this respect might be useful. The higher rate of pathogenicity in case of blastospores treatment could have been attributed due to the faster germination of the spores and the faster penetration of the cuticle as compared to conidiospores. There might be more chances of attachment of the conidiospores in the body cadavers of third instars larvae than that of earlier instars, therefore, infection may differ accordingly. In contrast to this, infection process might be more effective in early instars than the later instars because of the fast molting in the former cases than the later cases.

The considerable number of mortality obtained in untreated grub populations without any mycosed grubs may reflect a high natural mortality and/or suggests that some mortality may result from the conditions under which the grubs were kept. Future activities should include studies on the natural mortality as well as an improvement of the rearing methods.

## 5.6 CONCLUSIONS

Entomogenous fungi have great promise for use as biological control agents against different insects. However, their infectivity is quite different depending on fungus species and developmental stage of the target insects (Samson, 1981). Therefore, when a particular insect pest control programme is considered using these fungi, the particular species or strains which are most suitable have to be taken into account. Similarly, dose and time of exposure of the host to the insect pathogenic fungus and the time taken to kill the host are also important parameters for evaluating the suitability of insect pathogenic fungi. Fungi which need shorter exposure period and kill the host quickly are very important for practical application. Therefore, it is important that the activity of selected fungus isolates should be screened against the particular target host at the initial stage. Such pathogenic relationship may give ideas related to virulence of the species and to the number of fungus material necessary to kill at least 50% of the test population.

The screening experiment has convincingly showed all the isolates are pathogenic to white grubs, however, comparatively the isolates isolated from white grubs found slightly better than that of the isolates isolated from soil sources. Similarly, the dose related experiment showed that the higher dosages are more pathogenic than the lower dosage to induce mortality and mycosis. Nevertheless, the differences between $10^2$ and $10^8$ are only small. Most of the efficacy studies with entomopathogenic fungi for the microbial control of white grubs have been conducted on larval stages. Earlier investigations by Martins (1988) and Krueger *et al.* (1991) revealed the potential for augmentation or inundative treatments with *M. anisopliae* for the control of *Papoliae japonica* larvae. The infectivity of *M. anisopliae* against second instar larvae of *M. affinis* indicates good potential for controlling them while considering their biology. This experiment has further suggested that second instar grubs are more vulnerable as compared to other instars. Field application and mass production should be considered taking all these facts into full account for successful pest management.

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**Appendix 5.1**

**Detailed methods to count conidia of *M. anisopliae* using a Thoma haemocytometer for white grub bioassay experiments**

Haemocytometer counts of conidia on big square chamber of haemocytometer

= 108, 120, 83, 92

Average number of conidia on big square chamber = 403/4 = 100.75

The average number of conidia in the smallest unit area = 100.75/16 = 6.296 spores/ unit area

The area of the smallest unit of the haemocytometer (L x B) = 0.0025 $mm^2$ (this value is given on the Thoma haemocytometer)

Height of all units of the haemocytometer (H) = 0.100 m

Thus, the volume of smallest unit (L x B x H) = 0.00025 $mm^3$

L = Length, B = Breath, H = Height

Here, 6.296 spores/smallest unit of haemocytometer means 0.00025 $mm^3$ of suspension

OR, 0.00025 $mm^3$ of contains, 6.296 conidial spores

OR, 1 $mm^3$ of contains, 6.296/0.00025 conidial spores

OR, 1000 $mm^3$ of contains, 6.296/0.00025 x 1000 conidial spores

As 1000 $mm^3$ contains 1 ml of water

Therefore, 6.296 x 4 x $10^6$ spores/ml = 2.5184 x $10^7$ spores ml

# Chapter 6

## Effectiveness of indigenous and commercial fungi, *Metarhizium anisopliae* (Metsch.) and *Beauveria bassiana* (Bals.) against three species of white grubs under laboratory condition

# Chapter 6

# Effectiveness of indigenous and commercial fungi, *Metarhizium anisopliae* (Metsch.) and *Beauveria bassiana* (Bals.) against three species of white grubs under laboratory condition

## 6.1 SUMMARY

A total of eight different isolates of insect pathogenic fungi based on *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated in the bioassay experiments. Six isolates of *M. anisopliae* originated from white grub species from Nepal. An isolate each of *M. anisopliae* and *B. bassiana* originated from commercially formulated spore powder manufactured in India. Third instar larvae of three scarab species commonly present in maize fields in Chitwan and Nawalparasi (*Anomala dimidiata* Hope and *Adoretus lasiopygus* Burmeister) and in Tanahun (*Phyllognathus dionysius* F.) were assessed in a bioassay experiment in a completely randomized design (CRD) with nine treatments (including one control) and three replicates. The experiment was conducted with a single concentration of $1 \times 10^7$ spores/ ml using the dipping method, incubated at 20-22 $^oC$ in a dark room and checked regularly for 70 days. The experimental unit consisted of polypots with the supplement of slices of potatoes as food. Assessment parameters included mortality, infection and lethal time ($LT_{50}$). The experiments convincingly demonstrated that native isolates were more virulent ($p<0.001$) than exotic ones and this was evident across the tested white grubs species. On the other hand, the exotic isolates were able to induce a moderate extent of pathogenicity and they stood at par with other isolates of Nepalese origin. The methodological procedures for bioassay and possible reasons are explained in this chapter. Furthermore, this study has indicated that mass production can be done using virulent native strains for white grub control in particular and soil pests in general. Microbial control helps to protect the environment and contributes to the reduction of the use of harmful pesticides as envisaged in the integrated pest management (IPM).

## 6.2 INTRODUCTION

The pathogencity or virulence of a microbial insecticide is a function of many interacting factors and is relative to a specific set of conditions which exist between the microbial agent and a particular host. Virulence refers to the intensity of the disease caused by a pathogen, whereas pathogenicity refers to an organism's ability to cause disease. A fungal species which is pathogenic on a wide range of hosts may be more likely to persist in an environment because of the availability of alternate hosts. With a greater number of susceptible hosts, there may be a greater reservoir of inoculum available to produce an epizootic (Tanada, 1963). Virulence may be measured in a bioassay by exposing a known number of hosts to a known number of pathogens and observing the dead number over the time (Meynell and Meynell, 1965). The lethal time which produces mortality in 50% of the test hosts ($LT_{50}$) is generally used to compare virulence or the response of host death reasonably in a shorter period of time. The host range of *M. anisopliae* is wide exceeding 200 species of 7 orders of insects (Roberts and Yendol, 1971). The pathogenicity however varies with strains or isolates (Aizawa, 1987), pathogen population, dispersal and survival in the host's environment, and inoculum density and spatial distribution (Tanada and Fuxa, 1987). Selection of

effective strains of entomopathogens is essential for the development of microbial insecticides.

For the last few years, some of the indigenous strains of *M. anisopliae* were isolated from soil and white grubs within the white grub control research project of Helvetas/Switzerland and Nepal. In the meantime, dust formulation of *M. anisopliae* and *B. bassiana* based biopesticides are started to appearing in a few Nepalese shops who sell the chemicals for agricultural pest control and drugs for animal health (commonly know as agro-vets in Nepal). They were manufactured in India and been sold in agro-vets based in Kathmandu. The virulence of the indigenous isolates of *Metarhizium* was recently studied on larvae of *Maladera affinis*, while the effectiveness of introduced bioagents has not yet been known in an experimental basis. Therefore, comparative studies of the indigenous as well as exotic fungus strains to different pest species of white grubs common in the low land area of Nepal were carried and are described in this paper.

## 6.3 MATERIAL AND METHODS

### 6.3.1 Maintenance of indigenous fungus strains - *Metarhizium anisopliae*

The strains of *M. anisopliae* used in this experiment were isolated from white grubs. Six of the most aggressive fungal strains (M1, M6, M18, M48, M50 and M70) were obtained from the first tired experiment conducted during 2003-2005. The selective medium adapted from Strasser *et al.* (1997) with the following composition and preparation was used: 10 g Peptone from meat pancreatically digested, 20 g Glucose, 18 g Agar-agar, all dissolved in 1 l distilled water and autoclaved at 120 $^{o}$C for 20 minutes. At a temperature of 60 $^{o}$C; 0.6 g Streptomycin, 0.05 g Tetracycline and 0.05 g Cyclohexamide previously dissolved in distilled, sterile water were added. Each of the isolates was submitted to regular host passages using the natural hosts. They were transferred into sterialized Petriplates and test tubes. The isolates were incubated at 27±2 $^{o}$C and 80±5 % RH.

### 6.3.2 Maintenance of exotic fungus strains- *M. anisopliae* and *B. bassiana*

The cultures of the commercial formulations of *M. anisopliae* (Com Ma) and *B. bassiana* (Com Bb) were obtained from a non-governmental organization, International Development Enterprises (IDE) based in Kathmandu. Originally the head office had purchased these bioagents from agro-vets based in Kathmandu and was planning to test them against coffee pests in Syangja (western Nepal). The materials were obtained from such sources and stored in the normal refrigerator temperature until further use. The products were formulated as mycoinsecticides (50% WP) with 6 x 10 $^{9}$ spores/g. Inocula were prepared at the insect pathology lab, IAAS, Rampur based on its recommended dose of 1 g/l of water. For the bioassays about 30 ml fungal suspension containing 1 x 10 $^{7}$ spores / ml was used for dipping 30 larvae.

### 6.3.3 Maintenance of host insects - the white grubs

The grubs were collected from damaged crop field from different sites of Chitwan (230 m asl), Nawalparasi (175 m asl) and Tanahun (950 m asl). These include a number of farmer’s fields in Gunganagar, Saradanagar, veterinary block and mango orchard of

IAAS, Chitwan District and Gaindakot of Nawalparasi District. *Anomala* sp. and *Adoretus* sp. were mainly collected from Chitwan and Nawalparasi, and *Phyllognathus* sp. from Tanahun since the distribution of this species was location specific. The fields were dug beneath the crop root level and the grubs were picked up and placed individually in poly pots of 4.5 cm diameter and 6 cm height. Initially, all the recovered grubs were collected from the fields. Upon bringing them into the laboratory they were separated based on the instars and species with the help of larval keys. The larvae were transferred into the rearing vials supplemented with slices of potatoes. The soil as well as feeding materials was changed each week. Larvae were kept in quarantine at a temperature of 20-22 $^{o}$C for four weeks to reduce or avoid natural infestation. Naturally infected larvae were discarded and only non-infected larvae were used in bioassay experiments. The bioassay was carried out using conidiospores in a complete randomized design (CRD).

### 6.3.4 Production of fungus inoculum for bioassays

Isolates of *M. anisopliae* were maintained on SDA for no longer than two passages. Conidia were produced on the selective medium adapted from Strasser *et al.,* (1997). The conidia were harvested by scrapping off the contents of each Petri dishes or test tube with a sterile bacteriological loop. The conidial mass was dispersed in distilled water by using a drop of Tween 80 to overcome their hydrophobic nature. From the original concentration, 1 ml stock solution was drawn. The concentration was determined under a microscope using a Thoma haemocytometer and then adjusted to 1 x $10^{7}$ spores /ml as desired concentration. For the bioassays ten to 30 ml of the suspension were used for each strain to treat 30 larvae.

### 6.3.5 Assessment in bioassay experiment

The bioassay was carried out in two consecutive days, on 15 and 16 April 2005. Nevertheless, the inocula were harvested on the same day as the experiments were carried out. The bioassays were carried out using the dipping method (Goettel and Inglis, 1997) against third instars larvae of three different species of damaging white grubs such as *Anomala dimidiata, Adoretus lasipopygus* and *Phyllognathus dionysius*.

All bioassays were carried out with a concentration of $10^{7}$ spores/ ml. Larvae were dipped (plunged) individually for 3-5 seconds in the spore suspension. The drop of excess liquid was stripped off on a paper towel, the larvae were returned into the rearing container (cylindrical, inner diameter 45 mm, height 60 mm) filled 2/3 rd with sterile soil. The lid of the rearing pots was perforated to facilitate aeration. One group of white grubs was maintained as control just dipping in distilled water. All treated larvae were incubated at 20-22 °C in the dark. The 30 larvae were splitted into three replications with a batch of ten larvae per replication. Altogether there were nine treatments (6 indigenous strains, 2 exotic strains and 1 control) with three replications in a completely randomized design (CRD). The treated larvae were checked at weekly intervals and the experiment was conducted until 70 days at a temperature of 22-23 $^{o}$C. During the checks the larvae were fed with slices of potatoes and the sterile soil was moistened or replaced when felt as necessary. The dead larvae without signs of fungal infection were placed in a moist chamber (plastic Petri dish with a piece of moist filter paper) at 22 $^{o}$C for some additional days.

The observation parameters collected in this experiment were number of treated insect, number of dead insect and number of insects infected with *M. anisopliae*. From these data, $LT_{50}$ was calculated to identify the most virulent isolate. The mortality percentage was calculated from the total number of dead grubs to the total number of live grubs. In order to induce the mycosis and production of conidia, all the dead grubs were transferred to tubes containing wet soil. The proportion of mycosed grubs were calculated from the number of sporulating cadavers to the total number of grubs. The parameters were analyzed using GENSTAT-software computer package.

## 6.4 RESULTS

### 6.4.1 Assessment in bioassay experiment

The pathogenicity of different indigenous isolates of *M. anisopliae* as a well as commercial biopesticides with a concentration of $10^7$spores/ ml against third instars larvae of *Anomala dimidiata, Adoreturs lasiopygus and Phyllognathus dionysius* is presented in Table 6.1.

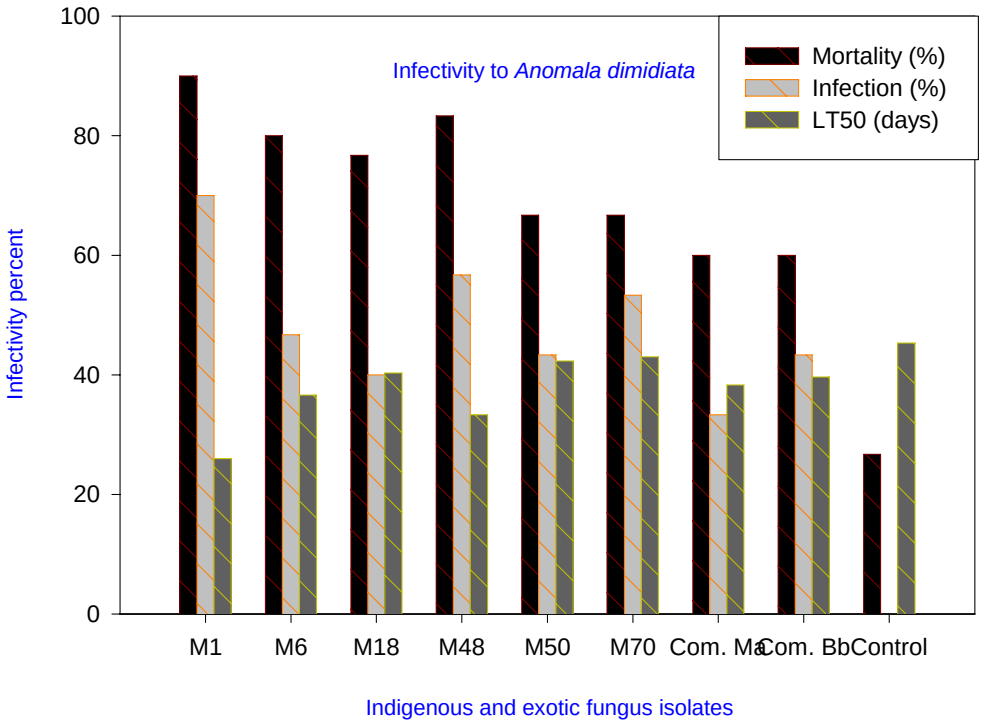
Table 6.1 Comparative study of the efficacy of indigenous and commercial strains of insect pathogenic fungi against three different species of white grubs (Mort = mortality and Infect. = infection).

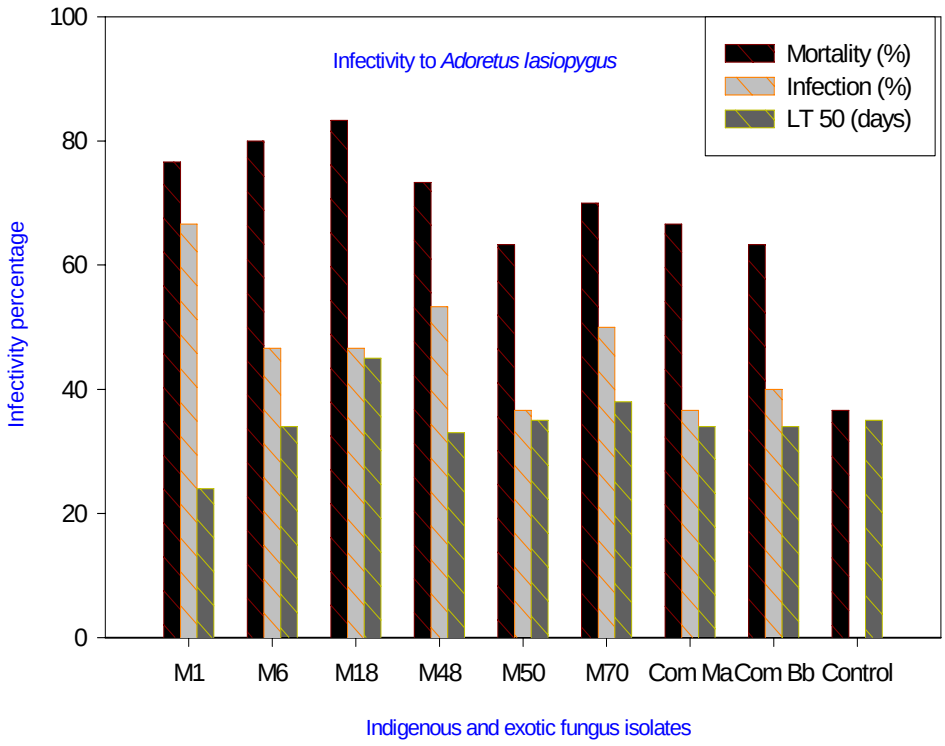
| Trt | Efficacy to three species of white grubs for mortality (%), infection (%) and LT50 (day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Anomala dimidiata* | | | *Adoretus lasiopygus* | | | *Phyllognathus dionysius* | | |
| | Mort. | Infect. | LT 50 | Mort. | Infect. | LT 50 | Mort. | Infect. | LT 50 |
| M1 | 90.0 d | 70.0 f | 26.0a | 76.6bc | 66.6d | 24.3 | 90.0 | 70.0c | 22.3a |
| M6 | 80.0 cd | 46.7 cde | 36.6ab | 80.0bc | 46.6bcd | 34.3 | 83.3 | 56.6b | 24.0a |
| M18 | 76.7 bcd | 40.0 bc | 40.3b | 83.3c | 46.6bcd | 45.3 | 76.6 | 50.0b | 26.3ab |
| M48 | 83.3 cd | 56.7 e | 33.3ab | 73.3bc | 53.3c | 33.3 | 80.0 | 56.6b | 29.0abc |
| M50 | 66.7 bc | 43.3 bcd | 42.3b | 63.3b | 36.6b | 35.3 | 86.6 | 53.3b | 28.6abc |
| M70 | 66.7 bc | 53.3 de | 43.0b | 70.0bc | 50.0bc | 38.3 | 90.0 | 56.6b | 28.3ab |
| Com. Ma | 60.0 b | 33.3 b | 38.3ab | 66.6bc | 36.6b | 34.0 | 80.0 | 50.0b | 31.0bc |
| Com. Bb | 60.0 b | 43.3 bcd | 39.6b | 63.3b | 40.0bc | 34.0 | 86.6 | 46.6b | 34.6bc |
| Control | 26.7 a | 0 a | 45.3b | 36.6a | 0.0a | 35.0 | 73.3 | 0.0a | 39.0d |
| Grand mean | 67.8 | 43.0 | 38.5 | 68.1 | 41.9 | 34.9 | 83.0 | 48.9 | 29.15 |
| SEM | 5.98 | 3.85 | 5.77 | 5.21 | 3.85 | 4.74 (ns) | 7.54 (ns) | 4.44 | 2.9 |
| LSD | 17.78 | 11.44 | 12.12 | 15.48 | 11.4 | 14.08 | 15.83 | 9.34 | 6.2 |
| CV % | 15.3 | 15.5 | 18.4 | 13.2 | 13.2 | 23.5 | 11.1 | 11.1 | 12.4 |

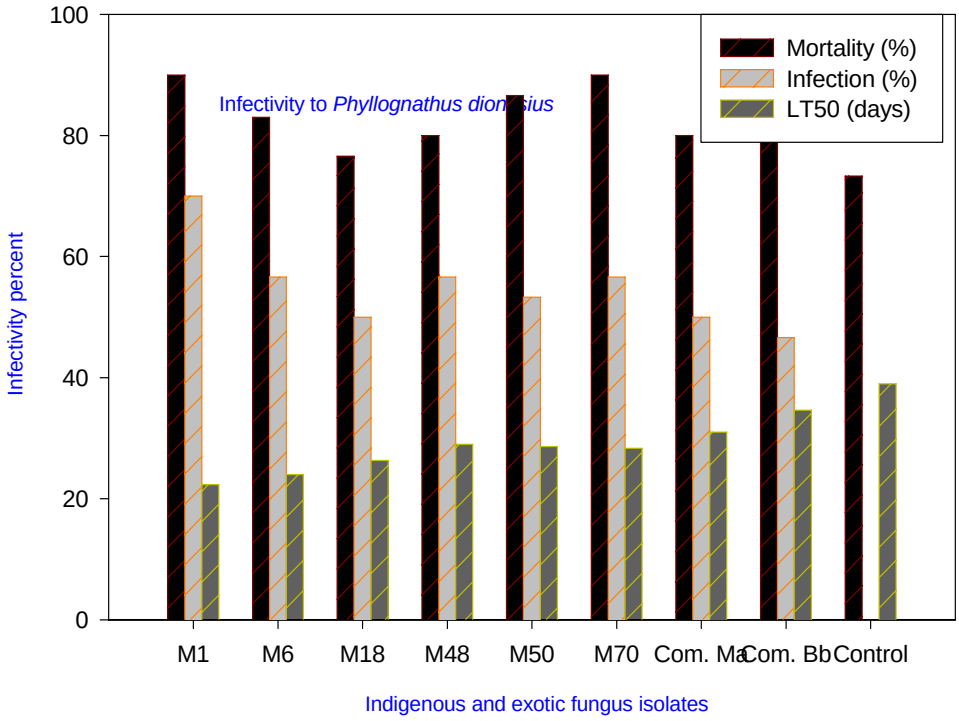
Figures in column followed by same letters are not significantly different at p<0.001 by DMRT

The experiment demonstrated that most of the indigenous isolates of *M. anisopliae* caused significantly higher mortalities and infection rates than the commercially

available *Metarhizium* and *Beauveria* formulations. This is evident not only in the case of a single species of Scarab larvae but found as a general trend across the tested species. Among the isolates, *Metarhizium* M1 was found to be the most virulent comparing to other isolates. It induced the highest proportion of mycosis as well as $LT_{50}$ in all the grub species. The pathogenicity of the treatments to different species of beetle larvae is presented in Figures 6.1-6.3.






Figures 6.1-6.3 Pathogenicity and virulence of different isolates of entomopathogenic fungi to three different species of scarab larvae. M1, M6, M18, M48, M50, M60: test isolates; Com Ma, Com Bb: Commercial isolates of *M. anisopliae* and *B. bassiana* respectively

Next to this, M48 and M70 also caused slightly higher infection rates as compared to the rest of the isolates. The efficacy of the exotic isolates was found at par with that of the rest of the native isolates. This finding is supported by earlier results which showed that conidiospores and blastosproes of *Metarhizium* M1 were equally virulent to the larvae of *Maladera affinis* as other isolates in a similar type of bioassay. Further, the data demonstrates that larvae of *P. dionysius* were more susceptible to the tested fungal isolates as compared with other white grubs species.

## 6.5 DISCUSSION

The bioassays have convincingly showed that some of the native strains are more virulent than the imported ones. A possible reason might be the loss of virulence during manufacturing, transportation and storage of the imported isolates. The exotic fungi were made by Multiplex Company of India and were stored in the agro-vet in the city centre of Nepal. The poor infectivity of these fungi could be due to the exposure to inappropriate temperature after their manufacturing. Longevity may consequently be lost during their storage without considering their required temperature. In general, they are stored at normal room temperature along with the general chemical compounds. In these areas, the temperature exceeds more than 25 $^{o}$C during the summer months which may cause detrimental effects on the viability of the fungus. There could be many possibilities for the higher virulence of native isolates as compared to exotic ones however, the fresh culture, duration of formulation and storage could have significantly affected for the virulence among the tested strains. Similarly, the type of inoculua might also have affected the virulence significantly. Other reason might be sources of isolation, storage condition, and duration of storage, target host and environmental

factors which should be taken into consideration while performing these kinds of experiments. This study has indicated the necessity for proper storing of the fungus materials after their formulations to until field application.

The reason for the higher susceptibility of *P. dionysius* larvae might have attributed due to the larger body size of these species as compared to the other two species. The life cycle of this species is relatively short as compared to the other two species; however, a relation to pathogenicity is not obvious.

The onset of higher rate of mortality and infection as induced by freshly cultured fungus isolates suggest the importance of proper storage and handling the fungus. Furthermore, the fungi isolated from insect host could have higher pathogenicity as compared to other strains isolated from other sources. The reason behind this is not clear, therefore, future activities should elaborate this effect.

Similarly, different responses of the fungi with respect to grub species might have governed by the body size of the grubs. The larger body sizes of grubs of *P. dionysius* provided more space to pick up conidia. Therefore, a larger number of conidia can be expected as compared to the grubs with smaller body sizes such as *A. lassiopygus* and *A. dimidiata.* Additionally, the cuticle of the former species is very soft and delicate as compared to the latter, which may allow more chances of penetration as compared to the latter species. In these species, the cuticle is very firm. Therefore, the chances to get infected may be higher in larvae of *P. dionysius* as compared to the other species. The variation of pathogenicity with regard to different strains could have been attributed also to fungus-specific characteristics. The considerable number of mortality obtained in untreated grub population without any mycosed grubs suggest that mortality may be induced by other factors like infections with other pathogens or sub-optimal rearing conditions.

## 6.6 CONCLUSIONS

The infectivity of native strains of *M. anisopliae* to different species of white grubs indicates good potential for further work of mass production without introducing exotic strains. This experiment has further suggested for the use of virulent strains while conducting mass production into a suitable substrates as a means to white grub control. Retention of the fungus virulence throughout production, storage and application of the fungus is very important. These fungi are very sensitive to higher temperatures; therefore, appropriate temperatures should be maintained during storage until application. The fungus material should be preserved at refrigerator temperature or inside a cool room for maintaining its virulence. Mass production, formulation, storage and field application are important factors which should be considered into full account for the larger scale production of fungus based biopesticides.

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# Chapter 7

## Mass production of the insect pathogenic fungus, *Metarhizium anisopliae* for field application against white grubs in Nepal

# Chapter 7

## Mass production of the insect pathogenic fungus *Metarhizium anisopliae* for field application against white grubs in Nepal

### 7.1 SUMMARY

Successful use of entomopathogenic fungi as microbial control agents of insects will ultimately depend on the use of the right propagule, formulated in an optimum fashion and applied in the field at the right time. In order to accomplish this, detailed knowledge of the host-pathogen interactions and epizootiology is necessary. Research and development must then concentrate on developing a method of mass production, formulation, and application that retains the ability of the pathogen to kill its hosts. Most research work, however, deals with the study of taxonomy, biology, and the pathogenic characteristics of these organisms whereas, techniques for the mass multiplication of fungi, practical formulations, and methods of application are less available. In order to address these issues, mass production of *Metarhizium anisopliae* was carried out considering some of the important parameters on the quality of the fungus materials. Comparative study of the fungus production has convincingly showed the marked difference ($p< 0.001$) between the Swiss polybags and Nepali polybags with a better quality of the fungus in the former types of bags. Similarly, barley kernels are found to be better substrates over the rice and wheat for fungus production. The fungus colonized barley grains originating from Nepali bags were heavily contaminated irrespective of the solid substrates used in the experiments. In the same way fungus propagules produced in the form of blastospores are found better than the conidiospores in terms of better quality fungus ($p<0.001$). In all cases, a total of five virulent strains of *Metarhizium anisopliae* were tested initially and M1 strain was found superior ($p<0.001$) amongst them. Each of the stages in the development and optimization of a mass production technique for the field research is discussed. The study has indicated the opportunity of producing fully colonized barley grains with the fungus as a means of controlling white grubs. This may serve as preliminary steps for commercialization of the fungus based biopesticides into the existing pest management in Nepal. Detailed producers of mass production adopted at the laboratory of the Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan of Tribhuvan University of Nepal has been explained.

### 7.2 INTRODUCTION

Mass production of conidia of entomopathogenic fungi for the biological control of insects is an area of growing interest (Bartlett and Jaronski, 1988, Bateman, 1997, Jenkins and Goettel, 1997). Recent development in solid state culture systems (Bradley *et al.* 1992) has presented the opportunity for industry to consider the production of entomopathogens in specially designed bio-reactors with automated process control. Such industrial production systems speed up the sporulation process by providing excess oxygen to the growing culture (Guillon, 1997) and, once produced, enable the conidia to be dried rapidly by forcing warm air at high velocity through the sporulated medium. In contrast, low technology mass production is also feasible in areas where the production process is slow, and in which drying of the sporulating fungus and the substrate occurs more slowly over several days, for example, a dehumidified room (Jenkins *et al.* 1998). The initial stages of

mycopesticide development begin with the collection of fungal isolates and screening for virulence to the target pest. Early in the development of any mycopesticide product there is a requirement for relatively small quantities of the fungus for both laboratory and field testing. The methods of production vary according to the experimental design and the stage of development within the objective of the study. For bioassays and general isolate screening, maintenance and production on agar slants in bottles is usually sufficient. As the study advances, one or more isolates have been selected for further development, greater amount of materials are required and it becomes necessary to demonstrate the feasibility of large-scale production (Jenkins *et al.* 1998).

The infective material of the fungus can be produced in two forms, either as blastospores in liquid media (submersed culture) or as conidia on solid media (surface culture). Both spore types exist in nature, the blastospores multiply in the haemolymph of the living host and the conidia develop on the surface of the dead host. Production can take place in liquid cultures, on solid media or using a combination of both. The type of fermentation system depends on the host to be treated. For aerial application a liquid product may be best, while a granule may be best for a soil treatment. Blastosproes are susceptible to environmental conditions and consequently less persistent than conidia (Aregger, 1992). Moreover, they are short lived and the host must be treated directly. In order to avoid this situation, more persistent conidia in a solid state would be more suitable to control the target pests. The submerged conidia are smaller than blastospores and thus theoretically producible in higher concentrations. Solid state fermentation is advantageous because it is easy to carry out, raw material is cheap, and most importantly, spores produced as living propagules tend to be more tolerant to desiccation and more stable as a dry preparation compared with spore produced in submerged fermentation (Deshpande, 1999). In general, artificial culturing of the fungus offers no particular difficulties; an utilisable source of carbon for germination, a nitrogen source for continuous hyphal growth (Smith and Grula, 1981), high humidity (80-100% RH), and temperatures between 2 and 25 $^{o}$C, with an optimum at 23-25 $^{o}$C (Ferron, 1978), are necessary. The production and formulation process should be followed by a quality control including purity of the product (foreign microorganisms, undesired metabolites) and virulence (Keller, 1998). Similarly, the application must be done carefully and directed against the target taking full account of its biology.

## 7.3 MATERIAL AND METHODS

In order to produce fungus material of high quality, initially five virulent isolates of *M. anisopliae* namely, M1, M6, M18, M48 and M50 were compared using different substrates and poly bags. The study was conducted in a complete randomised design (CRD) considering two factors each. Fungus isolates in one hand and solid substrates in the other hand were tested in one set of experiments whereas fungus isolates and poly bags were assessed in another set of experiments. Detailed description of each step is described below. The collected parameters were analysed using GENSTAT computer software package. The least significant differences (LSD) of means, standard error of differences (SEM) of means and the percentage coefficient of variation (CV) were mentioned for each parameter.

### 7.3.1 Collection of fungus inocula

With an objective to obtain indigenous fungus material, farmer's fields were surveyed in Parbat, Syangja, Tanahun, Chitwan and Nawalparasi since the middle of 2002. Soil samples and live insects were collected from the visited sites and fungus inocula were obtained and maintained at the Insect Pathology laboratory of IAAS, Rampur, Chitwan, Nepal using different methods (G. C. and Keller, 2002). Purity was maintained using host passages and final screening until the mass production was carried out. The selection of the isolates based on virulence tests as described in Chapters 5 and 6.

### 7.3.2 Preparation of fungus inocula and solid substrates

In mass production, two different types of inocula such as conidiospores and blastospores wereused. Conidiospores were prepared following the same methodologies as described in Chapter 6; however, blastospores were prepared using liquid medium. Liquid medium with following composition and preparation was used. This contains two solutions, solution A with 500 ml tap water 20 g Corn Steep, 2.26 g $KH_2PO_4$ and 3.8 $Na_2HPO_4$ and solution B with 500 ml tap water with 30 g Glucose. These solutions were autoclaved separately at 1.5 bar ($120^o$ C) for 40 minutes and later mixed together. Blastospores were prepared in such medium into flasks after shaking them at 100-120 rpm for 5-6 days. The process was conducted at $22^o$ C in UV protected room.

Peeled grains of barley, rice and wheat obtaining from local markets were tested as solid substrates. Similarly, the polybags of Swiss and of Nepal origin were used in fungus production. Swiss bags were polycarbonate bags with a size of 30 x 50 cm, whereas Nepali bags were of the same size but prepared for general use of carrying grocery items from the shops. Swiss bags were obtained with a kind support from Dr. Siegfried Keller, FAL Agroscope Reckenholz, Switzerland and Nepali bags were purchased from local market.

Before inoculation of the fungus, one kg solid substrate was filled in a poly bag. 300 ml normal tap water was added and left for four hours before autoclavation for uniform soaking of the kernels. The substrates were autoclaved twice at an interval of 24 hours at a pressure of 1.5 bars which is equivalent to 120 $^oC$ for 40 minutes. Fungus inocula consisting of conidiospores or blastospores were adjusted to $10^7$spores/ml in a Thoma haemocytometer under a stereomicroscope. After proper cooling of the grains, inoculation was conducted in a laminar flow hood.

### 7.3.3 Inoculation of solid substrates with blastospores

Each flask containing the blastospore suspension was diluted 1:1 with the addition of 100 ml of sterilised tap water. The fungus inocula were well shaken to get a homogenous suspension. The bags with the sterilised medium were carefully opened at one corner and 200 ml diluted blastospore suspension per 1 kg solid substrates was inoculated. In order to assure a homogenous distribution of the inocula in the solid substrates the poly bags were shaken very gently before incubation.

### 7.3.4 Incubation of inoculated solid substrates

The bags were incubated in a temperature regulated incubator at 22-23 $^{o}$C for more than two weeks. In order to favour the homogenous growth of the fungus on the kernels, to improve aeration and to prevent the kernels from sticking together, the bags were shaken daily during the initial days of inoculation. The bags were put in an upside fashion of the openings to guarantee the air exchange. Similarly, piling up of the bags one over another was avoided to minimise the chances of heating during fungus growth. After three to four weeks, the bags with colonised kernels were removed from the incubator and air dried for storage on boards at a temperature of 22 $^{o}$C before utilisation. None of the bags were stored more than three months of its preparation. In all steps, bags were handled with care to avoid the damages that may lead to contamination.

### 7.3.5 Quality control of the fungus materials

Based on the virulence of the fungus isolates, *M. anisopliae* M1 was promoted for mass production. Assessment of the fungus material was carried out with respect to the type of fungus inoculum (conidiospores and blastospores), type of poly bags (bags of Swiss and Nepali origin) and the solid substrates (peeled kernels of barley, rice and wheat). The quality of the bags was checked in terms of proper growth and purity of *M. anisopliae.* Visually, they can be observed as white mycelium followed by green conidia; therefore, the contaminated bags without such qualities along with smelling bad odours were rejected. The quality of the fungus material in all the observation parameters was assessed with four different sets of observations as repetition of the experiments. The experimental unit in all studies was a Petri plate with moistened filter paper with fifty randomly selected fungus colonised kernels incubated at 22 $^{o}$C for four weeks. Eventually, quality of the fungus materials was expressed in terms of percentage colonised kernels and attributed to three groups: pure *Metarhizium* growth, *Metarhizium* present on a part of the grain with or without unwanted fungi (contaminants) and grains only with contaminants. In general, fungus quality was assessed in terms of the effects of the type of inocula, poly bags and solid substrates.

## 7.4 RESULTS

### 7.4.1 Collection of fungus strains

Since the mid of 2002, a total of seventy different strains of green muscardine fungus (*M. anisopliae*) and eight different strains of white muscardine fungus (*B. bassiana)* were recovered from different sources. *Metarhizium* were obtained from white grubs (24), from soil using *Galleria* Bait Method (GBM) (41) and from the soil using selective medium (SM) (5) Similarly, among eight *Beauveria* strains 6 were isolated from soil sources using GBM and only 2 from a white grub (Table 7.1).

It is interesting to note that the fungus strain used in the mass production study was originally isolated from a larval *Xylotrupes gideon* collected from Parbat District (1120 m asl) of Nepal in May 2002. In order to better handling the strains were later named with M1 to M70 for *Metarhizium* strains and B1 to B8 for *Beauveria* strains.

Table 7.1 Origin of the insect pathogenic fungi isolated 2002-2005 from different localities in Nepal. GBM: *Galleria* bait method; SM: selective medium

| Fungus species | Geographic origin | Number of isolates | Isolated from | | |
|---|---|---|---|---|---|
| | | | White grubs | Soil/GBM | Soil/SM |
| *M. anisopliae* | All | 70 | 24 | 41 | 5 |
| | Chitwan | 25 | 10 | 15 | 0 |
| | Parbat | 35 | 7 | 23 | 5 |
| | Tanahun | 7 | 4 | 3 | 0 |
| | Nawalparasi | 3 | 3 | 0 | 0 |
| *B. bassiana* | All | 8 | 2 | 6 | 0 |
| | Chitwan | 7 | 2 | 5 | 0 |
| | Parbat | 1 | 0 | 1 | 0 |
| | Tanahun | 0 | 0 | 0 | 0 |

The natural infection of white grubs with *M. anisopliae* was found to be low however; it seemed to be wide-spread in the soils of Nepal as compared to *B. bassiana*. This result has convincingly showed that the *Galleria* bait method is very suitable for obtaining the entomopathogenig fungi from soils and in a reasonably shorter period of time. In addition, it demands low sophisticated techniques and knowledge compared to other methods. These master isolates so far recovered are preserved at IAAS, Rampur, Chitwan, Nepal and Agroscope FAL Reckenholz, Zurich, Switzerland and serve as material for further works in this regard.

### 7.4.2 Preparation of fungus inocula and solid substrates

Fungus inocula were prepared following the same methodologies as described in Chapter 5. Production of blastospores of *M. anisopliae* in liquid medium using a rotary shaker was successfully achieved within about a week. Similarly, different amounts of water resulted in different quality of the fungus. Addition of water to solid substrates is somehow a tricky thing which largely depends on practice and may not be applicable as rule of thumb while working with all types of solid materials. In general, most of the observation has proved that barley kernels gave consistent results and found to be less sticky at a 1:1 ratio of liquid and solid materials compared to rice and wheat. The autoclavation temperature of 120 $^{o}$C for 40 minutes did not cause contamination problems.

### 7.4.3 Quality control of the fungus materials

The comparison between inocula based on conidiospores and blastospores on the quality of the fungus material is presented in Tables 7.2 and 7.3. Table 7.2 shows that isolate M1 produced comparatively less uncolonised grains and a higher percentage of pure *Metarhizium* grains as compared to the other isolates when inoculated with blastospores, however, the differences are not significant.

Table 7.3 clearly shows that inoculation with blastospores of isolate M1 resulted better fungus quality than inoculation with conidiospores. This study has shown that isolate M1 is suitable for future mass production.

Table 7.2 Effect of different isolates of *M. anisopliae (M. a.)* and inocula types on the quality of fungus colonized barley grains.

| Strains | Conidiospore treated grains (%) | | | Blastospore treated grains (%) | | |
|---|---|---|---|---|---|---|
| | With pure growth of *M. a.* | With presence of *M. a.* | With uncolonized *M. a.* | With pure growth of *M. a.* | With presence of *M. a.* | With uncolonized *M. a.* |
| M1 | 82.50 | 69.50 | 30.50 | 95.00 | 99.55 | 4.50 |
| M6 | 84.50 | 64.00 | 36.00 | 87.00 | 95.00 | 13.00 |
| M18 | 77.00 | 64.00 | 36.00 | 89.50 | 97.50 | 10.50 |
| M48 | 79.00 | 67.00 | 33.00 | 93.00 | 96.50 | 7.00 |
| M50 | 82.00 | 67.00 | 28.00 | 90.50 | 97.50 | 9.50 |
| LSD (5%) | Ns | Ns | Ns | ns | ns | ns |
| SEM | 3.36 | 3.12 | 3.16 | 3.12 | 3.36 | 3.16 |
| CV % | 7.60 | 8 | 30.40 | 8.00 | 7.60 | 30.40 |

Table 7.3 Effect of fungus inocula of isolate M1 on the quality of fungus colonized barley grains inoculated at 22-23 $^{o}$C *M.a. = Metarhizium anisopliae*

| Spores | % grains with pure *M.a* growth | % grains with *M.a* present | % grains uncolonized with *M.a* |
|---|---|---|---|
| Conidiosproes | 66.30 | 81.00 | 32.70 |
| Blastospores | 91.00 | 97.20 | 8.90 |
| LSD (5%) | 4.038 | 4.35 | 4.08 |
| SEM | 1.398 | 1.50 | 1.41 |
| CV % | 8.0 | 7.6 | 30.4 |

It is obvious from Table 7.3 that the inoculums produced from blastospores are far more effective with that of conidiospores while planning mass production. The possible reason for this may be the effective growth of germinating spores in the former cases, whereas considerable extent of losses of conidia in later cases.

This study has clearly demonstrated that the blastospores are able to produce pure and heavily colonised *Metarhizium* kernels than the inoculation with conidiospores ($p<0.001$) (Figure 7.1).

The production of isolate M1 in two different types of poly bags revealed that, M1 strain produced comparatively pure *Metarhizium* fungus ($p<0.001$) among the tested strains (Table 7.4).

Figure 7.1 Barley kernels colonized with *M. anisopliae*

Table 7.4 Effect of fungus strains with respect to poly bags on the quality of fungus in IAAS, Rampur, Chitwan, Nepal (22-23 $^{o}$C) in 2005. *M. a.* = *Metarhizium anisopliae*

| Strain | Fungus quality in Swiss bag | | | Fungus quality in Nepali bag | | |
|---|---|---|---|---|---|---|
| | % grains with pure *M.a.* growth | % grains with *M.a.* present | % contaminated grains | % grains with pure *M.a.* growth | % grains with *M.a.* present | % contaminated grains |
| M1 | 98.00 | 100.00 | 2.00 | 53.00 | 72.50 | 18.00 |
| M6 | 91.00 | 99.00 | 8.00 | 67.00 | 92.50 | 25.50 |
| M18 | 90.50 | 97.50 | 6.50 | 27.00 | 47.00 | 20.00 |
| M48 | 83.00 | 97.50 | 14.50 | 62.50 | 89.50 | 27.00 |
| M50 | 90.00 | 99.00 | 9.00 | 15.00 | 24.00 | 9.00* |
| LSD (5%) | 27.04 | 40.10 | 15.67 | 27.04 | 40.10 | 15.67 |
| SEM | 9.36 | 13.88 | 5.43 | 9.36 | 13.88 | 5.43 |
| CV % | 27.70 | 33.90 | 77.80 | 27.70 | 33.90 | 77.80 |

* In this case rest of the 52% grains were found uncolonised by the fungus * In this case rest of the 52% grains were found uncolonised by the fungus

Based on this study, it is straightforward evidence that this strain may be promoted for further production scheme along with Swiss types of poly bags (Figure 7.2)

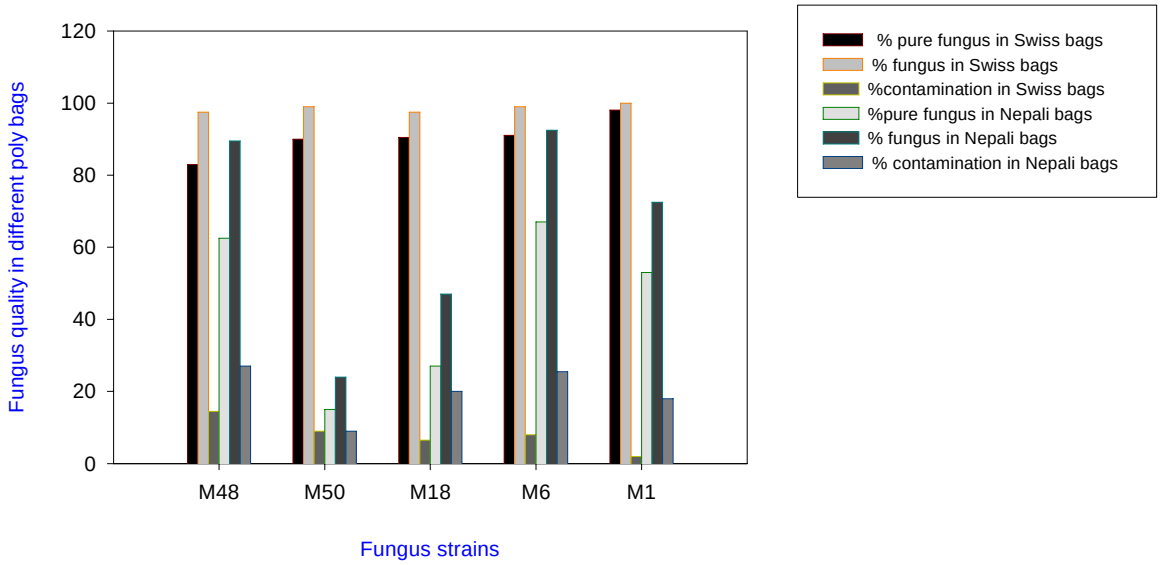


Figure 7.2 Effect of *M. anisopliae* isolates with respect to poly bags on the quality of fungus inoculated at 22-23 $^{o}$C.

While considering the effect of poly bags on the fungus quality, Swiss bags were significantly better (Table 7.5) than the bags of Nepali origin. The fungus colonized barley grains originating from Nepali bags were heavily contaminated with other unwanted saprophytic fungus when compared with the fungus material produced in Swiss bags ($p<0.001$) (Figure 7.3).

Table 7.5 Effect of poly bags on the quality of fungus colonised barley grains incubated at 22-23 $^{o}$C. *M.a. = Metarhizium anisopliae*

| Type of poly bags | % grains with pure *M.a* growth | % grains with *M.a.* present | % grains uncolonized with *M.a* |
|---|---|---|---|
| Swiss Bag | 90.5 | 98.6 | 8.0 |
| Nepali Bag | 44.9 | 65.1 | 19.9 |
| LSD (5%) | 12.09 | 17.93 | 7.01 |
| SEM | 4.19 | 6.21 | 2.43 |
| CV % | 27.7 | 33.9 | 77.8 |

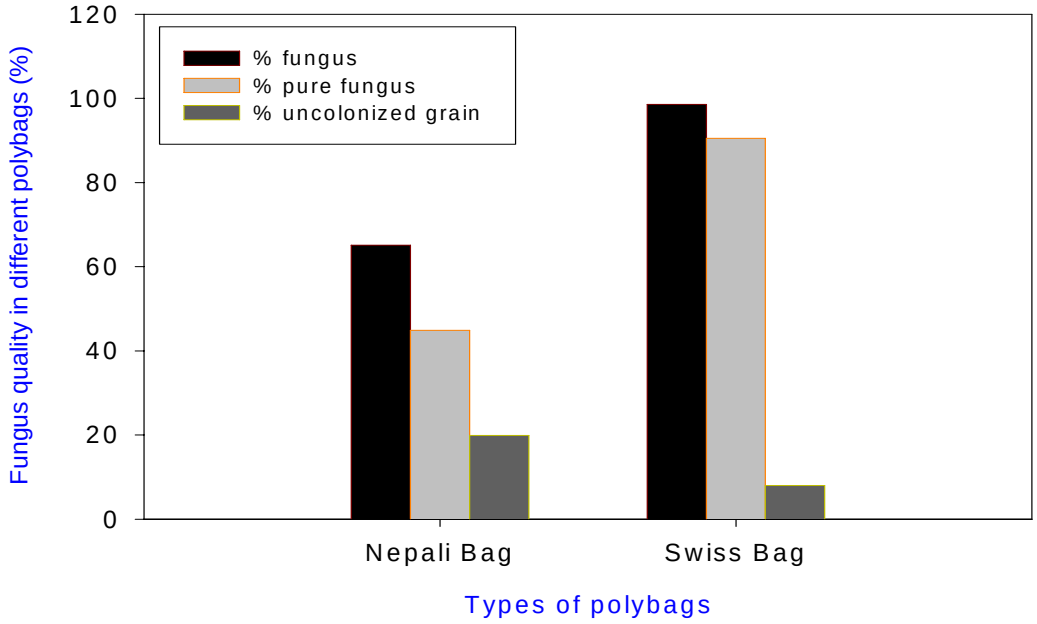


Figure 7.3 Effect of poly bags on the quality of fungus colonised barley grains incubated at 22-23 $^{o}$C.

The quality of the fungus in terms of purely colonised grains found significantly different ($p<0.001$) between two types of poly bags (Table 7.6).

Table 7.6 Effect of poly bag types with respect to solid substrates on the quality of M1 isolate of *Metarhizium anisopliae* (*M. a.)* inoculated at 22-23 $^{o}$C.

| Test substrates | Fungus quality in Swiss bags (%) | | | Fungus quality in Nepali bags (%) | | |
|---|---|---|---|---|---|---|
| | Grains with pure *M. a.* growth | Grains with *M. a.* present | Contaminated grains | Grains with pure *M. a.* growth | Grains with *M. a.* present | Contaminated grains |
| Barley | 94.5a | 99.5a | 5.0a | 53.5b | 74.0b | 20.5a |
| Wheat | 25.0b | 63.0b | 38.0b | 10.0a | 40.5a | 30.5ab |
| Rice | 38.0b | 61.0b | 23.0b | 32.5ab | 69.5b | 33.5ab |
| LSD (5%) | 11.58 | 30.99 | 23.52 | 11.58 | 30.99 | 23.52 |
| SEM | 3.9 | 10.43 | 7.92 | 3.90 | 10.43 | 7.92 |
| CV % | 18.4 | 30.7 | 63.1 | 18.4 | 30.7 | 63.1 |

Figures in the columns followed by same letters are not significantly different at $p<0.001$ by DMRT

The Swiss bags were found as a better production tool for fungus colonized cereal grains since prominent differences were observed in all three quality groups. The quality of the fungus in terms of uncontaminated grains was significantly higher in case of barley kernels produced in Swiss bags (Figure 7.4) as compared to Nepali bags. The fungus quality with references to different solid substrates (cereal grains) is presented in Table 7.7.

Table 7.7 Effect of solid substrates on the quality of fungus colonized grains inoculated with *Metarhizium anisopliae (M. a.)* isolate M1 at 22-23 $^{o}$C.

| Solid substrates | Observation parameters | | |
|---|---|---|---|
| | Grains with pure *M. a.* growth | Grains with *M. a.* present | Contaminated grains |
| Barley | 74.0 a | 86.8 a | 12.7 c |
| Wheat | 17.5 c | 51.8 b | 34.2 a |
| Rice | 35.2 b | 65.2 b | 28.2 a |
| LSD (5%) | 8.19 | 21.91 | 16.63 |
| SEM | 2.76 | 7.38 | 5.6 |
| CV % | 18.40 | 30.70 | 63.1 |

Figures in the columns followed by same letters are not significantly different at p<0.001 by DMRT

Table 7.7 shows the role of solid substrates in terms of higher or lower rate of contamination which eventually is related to the quality of the fungus. The lowest contamination rate (12.7%) was obtained when the fungus was produced on barley grains (p<0.001), followed by rice. The highest contamination rate (34.2%) when the fungus was produced on wheat grains (Figure 7.4).

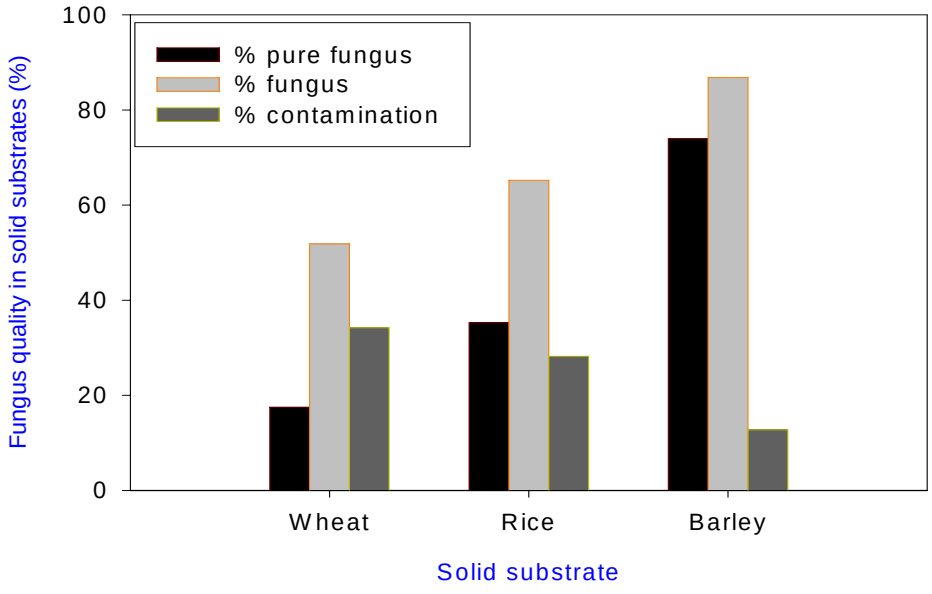


Figure 7.4 Effect of solid substrates on the quality of *M. anisopliae* isolate M1 produced at 22-23 $^{o}$C.

## 7.5 DISCUSSION

The entomopathogenic fungi *M. anisopliae* and *B. bassiana* were recorded from Nepal for the first time. Earlier (G. C. *et al.* 2004) it was demonstrated that especially *M. anisopliae* is widely distributed in soils from low to high hill regions, in cropland as well as in grassland. However, *B. bassiana* is poorly distributed in soils as compared to *M. anisopliae.*

The bioassays with the fungal isolates demonstrated that virulence differed among the strains and showed that isolates from white grubs are not *a priori* more virulent than isolates originating from soils. The *Galleria* bait method (GBM) proved to be a suitable method to detect the presence of *M. anisopliae* and *B. bassiana* in the soils. The bioassays further demonstrated that more strains of the known insect pathogenic fungi can be found with good potential for white grub control. Although further white grub pathogenic species like *B. brongniartii* may be found in future investigations in Nepal, the efforts to develop a mycoinsecticide are focused on *M. anisopliae.*

The experiments for mass production with *M. anisopliae* in autoclavable plastic bags demonstrated the feasibility but also the importance of the bag quality. Good fungus quality was only achieved with bags of Swiss origin and used therefore many years (Keller, 2004). Additional efforts must be undertaken to develop a production system which is based only on materials available on the national market. Mass production experiment has indicated ample opportunity of fungus production using barley kernels in suitable poly bags allowing exchange of air. During observation, it was found that the influence of bags for the contamination. In Nepali bags most contaminants were yeasts like with a typical smell, while the contaminants in the Swiss bags were *Penicillium*-like fungi. These marked differences between the two bag types are attributed to material characteristics. It is assumed that the Nepali bags are air-tight while the Swiss bags allow some gas exchange and evaporation of water.

Hand inoculation with liquid medium to autoclaved barley grains under laminar flow hood could be one of the appropriate methods for mass production for small scale production. However, in large scale production, some of the mechanised methods could be necessary. Due care should be considered for maintaining a sterile environment since the blastospores are highly prone to be contaminated with other unwanted fungi.

The interaction of solid substrates with respect to poly bags was not significantly different. However, the intensity of contamination was comparatively low in case of Swiss poly bags (5 and 20% contamination respectively). The possible reason for this may be the low stickiness of the barley grains after autoclavation as compared to other solid substrates. It was also found that the wheat grains were the poorest material for producing quality fungus where the highest contamination (38%) was found even in the Swiss bags. In case of unavailability of barley kernels, low quality rice could be another choice for using solid substrates in fungus production.

It is also important to reflect about other methods of mass production and other types of fungus formulation like production in liquid media and spore powder formulations. Changes in the production system may improve the availability of local materials, the risk of less contamination may be reduced and the yield of produced fungus increased. Therefore, future efforts are necessary to adapt the production system to the local resources and to the needs of the producers and the farmers. Production and application techniques should essentially be compatible with the availability of the materials under the local conditions.

## 7.6 CONCLUSIONS

Insect pathogenic fungi are available in Nepalese soils and also associated with insect populations. Among them, *M. anisopliae* is widely distributed than *B. bassiana.* It can be isolated and cultured on selective medium; mass production can be done by growing the fungus in a liquid medium which is used to inoculate locally available cheap solid substrates such as barley grains. Blastospores proved to be a better inoculum than conidiospores. The type of fungus production bags has a strong influence on the fungus quality and consequently on yield. Bags of Swiss origin gave better results than the Nepali bags. Future large scale production may be carried out using this as a foundation work. At the same time it is important to investigate different methods of production and formulation techniques to fulfill the farmer's requirements. It is hoped that such material can be produced and manufactured locally and made available to the farmers for field application. These soil fungi can be produced and applied as a means to white grubs control in particular and soil insect pest in general through inoculative and inundative release with the joint efforts of research, extension and private counterparts.

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# Chapter 8

## Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* as biocontrol agent against white grubs under field conditions in Nepal

# Chapter 8

## Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* as biocontrol agent against white grubs under field conditions in Nepal

### 8.1 SUMMARY

White grubs are increasingly becoming difficult pests in Nepal in the last few years. Controls of these pests were largely abandoned in the majority of the farming situation because of the lack of control options. Indiscriminate use of chemical pesticides at the farmer's discretion and accessibility are also common in some of road access areas. These latter practices have further aggravated the pest problem resulting in a wider reluctance to the cultivation of major cereals as well as cash crops in upland farming. With a view to address these issues alternative control measures based on the fungal antagonist *Metarhizium anisopliae* in Nepal at field level was a first ever attempted microbial approach in the country.

The results of the field experiments revealed that the fungus can infect the grubs, however, the infection rates were very small (15-20%) and this parameter remains insignificant when tested at different dosages. In contrast to the infection rates, the densities of the fungus after inoculation into the soils was found highly significant ($p<0.005$) since the colony forming units (CFU) differed greatly when compared before and after application. In general, both the infection rate and fungus density remained effective for three to four months but did not last at the same extent until crop harvests. This result has clearly demonstrated that several factors are responsible for the spread of the fungus in the soil. In this experiment, the host density and the soil temperature might have attributed markedly to the result since the grub density was reduced until three months after sampling. In addition to these factors, the virulence of the BCAs might play a great role depending on the method and season of application and assessment. From this experiment, it can be summarised that *M. anisopliae* can be applied as an important component of white grub management with some modifications. We have to improve factors like method of production of the fungus, its handling, and timing and frequencies of application. In order to mirror the laboratory findings into field conditions, further research is necessary to investigate the field efficacy of the fungus as biocontrol agent in the coming seasons. This was the first attempt towards microbial control of soil born pests in Nepal, where many aspects of biocontrol are still in rudimentary stages. The persistence and survival of the fungus in the soils are other aspects which need to be considered in further studies.

### 8.2 INTRODUCTION

White grubs are among the difficult pest insects in Nepalese agriculture. The complexity of the white grub's problem in Nepal is also aggravated by the fact that various species are involved depending on season and locality. The experiments of the past two years on population dynamics showed that *Maladera affinis* Blanchard, *Anomala dimidiata* Hope and *Adoretus lasiopygus* Burmeister are dominantly involved in maize growing area of the lower belts of Nepal whereas *Phyllognathus dionysius* F., *Holotrichia* spp, *Xylotrupes gideon* L. and *Lepidiota albistigma* Burmeister dominate in mid to high hills of Nepal. The

adults of these species swarm during April-May and sometimes continue until monsoon seasons. The larvae, also called white grubs, pass three larval instars (L1-L3) and stay in the soil. The life cycle of the white grub species existing in the terai (tropical) areas of Nepal is short as compared to the mid and high hill areas. Because of this, some of the species like *Maladera thomsoni* can be observed annually and in majority of the cases they occur in two peaks of the year whereas other species such as *L. albistigma* occur in alternate years. In addition to this, the damage in tropical belts of Nepal is also the result of the involvement of numerous species whereas in the mid hills, some crop may escape from damages because of the alternate years of pest occurrence. The larval stage of biennial species lasts nearly a year, whereas pre-pupal, pupal and adult stages take another year. In addition to this phenomenon, sometimes no attack takes place for a whole year but heavy damages occur in the following years. Nevertheless, the soil inhibiting larvae feed on roots and cause heavy damages mainly to perennial crops and in grassland (Keller 1986, Vestergaard *et al.* 2002) mainly after the year of massive flights.

There is a common trend of using highly hazardous chemical pesticides against soil pests in Nepal. Some of the banned insecticides such as phorate are still used in carrot and potato growing areas of Chitwan and Makawanpur Districts of Nepal (Field observation). Because of the impact of INGOs, farmers in recent years have realised their associated hazards and reduce the use of chemical pesticides. This has stepped up of the exploitation of natural enemies such as bio-control agents. Insect pathogenic fungi like *M. anisopliae* are a potential microbial alternative to chemical pesticides and offer a number of benefits such as facility of growth on a variety of substrates, high virulence, transcuticular penetration, broad host ranges, and safety to humans, animals and the environment (McCoy, 1977). These fungi play a significant role in the regulation of insect populations in nature and may potentially be exploited for commercial biological control purposes (Bidochka *et al.* 1995). They are used as mycoinsecticides for soil insect control in several countries, especially in USA, New Zealand, Australia, Switzerland, Austria and to some extent in Italy under different trade names (Butt *et al.* 2001).

Keller (2000) reported that a product based on sterilised barley kernels colonised with *B. brongniartii* proved to be a suitable and economically reasonable BCA to control *M. melolontha.* The product is commercialised in Switzerland since 1991. The success of the control of soil born pests with entomopathogenic fungi is mainly dependent on the efficacy and persistence of the pathogen in the soil environment coupled with soil temperature and moisture (Studdert *et al.* 1990), soil types (Storey *et al.* 1989) as well as with antagonistic organisms (Lingg and Donaldson, 1981). Another important factor is the presence of the host, which affects the persistence of mycopathogens in the soil (Kessler, 2004). Dispersal of spores is achieved by soil water, the movement of soil organisms and of infected and uninfected hosts (Hall and Dunn, 1957). Therefore, knowledge of the survival of the BCA in the soil is an important aspect in the process of developing and improving biological control strategies (Kessler, 2004). Considering all these aspects, a product based on sterilized barley kernels colonized with *M. anisopliae* was developed at the Insect Pathology Laboratory of IAAS, Rampur, Chitwan, Nepal since 2004. The present study investigates the efficacy of different dosages of indigenous isolates of *M. anisopliae* against white grubs and the development of the inoculum in maize based farming as the first field experiment in the country.

## 8.3 MATERIAL AND METHODS

### 8.3.1 Experimental sites

The study sites were selected based on the increasing infestation of white grubs which was observed by own samplings or reported by local inhabitants. White grubs problem in these sites were also reported by District Agricultural Development Offices (DADO). The experiments were conducted at four different sites. The first two sites were located in Chitwan District, one in Gunganagar and another in Saradanagar. The third and the fourth sites were located in Gaindakot (Nawalparasi District) and Nahala (Tanahun District). All the sites were located in farming areas. The location of the study sites is presented in Figure 8.1.


Figure 8.1: Map of Nepal showing the fungus application research sites (1 = Gunganagar; 2 = Saradanagar; 3 = Gaindakot; 4 = Nahala) in 2005.

### 8.3.2 Land preparation and soil samplings

In flat land (terai area), the land could plowed twice using tractor operated moldboard plough followed by harrowing and pulverizing the soil clods. After this, the research plots were prepared adopting farmers practices using bullock operated plow. The weed was removed during the plowing operations and picked up later manually. The plots were fertilized using well decomposed farm yard manure (FYM) at the rate of 1 t/ha. FYM was incorporated inside the soil after plowing the lands (Figure 8.2). No chemical fertilizers were applied in plots where the fungus application was carried out. The study sites were divided into four plots of 10 m x 10 m size per treatment leaving two meter border area between the treatments. Soil samples were obtained using a cylindrical soil sampler with an inner diameter of 5.5 cm. The soil was taken from a depth of 10-20 cm.

Figures 8.2 Preparation of experimental plot

Figures 8.3 Grubs sampling in experimental plot

The samplings (Figure 8.3) were taken in three consecutive frequencies i. e. initial (immediately before fungus application, mid (during half of the period of fungus

application) and final (immediately after the crop harvest). In this experiment the date of fungus application in different sites was different (Table 8.1). It is because, the maize sowing in Nepal depends on the monsoon however, the samplings for each site were maintained as uniform at each sampling frequencies. The initial sampling was taken before 1 week of fungus application, mid sampling after 7 weeks of fungus application and final sampling after 13 weeks of after fungus application. At each sampling period six soil samples were taken from a plot.


Fig 8.4 Participant farmers in Gunganagar, Chitwan research site

The soil samples that could not be analyzed immediately were stored in plastic bags at 4$^{o}$C not longer than 3 weeks at refrigeration temperature until they were used. The detailed information on experimental plots including date of fungus application, soil factors such as pH, content of clay, sand, silt; loam and moisture are presented in Table 8.1. The soil properties were analyzed at the central laboratories of the Institute of Agriculture and Animal Sciences (IAAS) Rampur, Chitwan, Nepal. Prior to 1 week of fungus application, observation parameters such as soil characteristics, soil samples for determining the initial density of fungus, density of beetle larvae were taken as initial sampling. After taking the initial samples, the experiments were set up in the field of participating farmers as shown in Figure 8.4. All plots were separated from each other by 2 m wide stripes. In the same way, every replication was separated by a terrace to avoid intra-plot effect of fungus and possible movement of the beetle larvae. The maize seeds and fungus kernels were placed together in the furrow depths made by bullock drawn plough. In order to sow the fungus, one person made the furrow with the help of bullock, second person put the maize seeds and third person placed the fungus kernels in the same row.

Table 8.1 Location of field trial sites sowing crops, date of fungus application, soil properties, soil moisture, pH and temperature (All fields were sown with maize crop).

| Location of trial sites and region | | | Date of fungus application | Soil parameters # | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Geographic coordination | Altitude (m asl) | | Moisture* (%) | | | Percentage of the contents | | | | pH | Temperature ($^{o}$C) | | | | |
| | | | | Initial | Mid | Final | Sand | Silt | Clay | Overall ranking | | March | April | May | June | July |
| Gunganagar | Central 27$^{o}$39$^{l}$N, 84$^{o}$19E | 230 | 24 March 2005 | 15.8 | 18.8 | 24.0 | 76.7 | 11.8 | 11.9 | Sandy loam | 5.0 | 25.2 | 29.3 | 30.4 | 31.0 | 30.6 |
| Saradanagar | Central 27$^{o}$38.5 84$^{o}$22E | 230 | 29 March 2005 | 15.2 | 18.7 | 24.9 | 71.9 | 15.6 | 12.3 | Sandy loam | 5.7 | 25.2 | 29.3 | 30.4 | 31.0 | 30.6 |
| Gaindakot | Central 27$^{o}$42.5 84$^{o}$25E | 205 | 4 May 2005 | 16.8 | 19.0 | 25.8 | 72.2 | 13.3 | 14.4 | Sandy loam | 6.0 | 24.6 | 28.7 | 28.8 | 30.0 | 30.2 |
| Nahala | Western 28$^{o}$11.5$^{l}$N, 83$^{o}$2 | 905 | 22 May 2005 | 18.2 | 24.0 | 26.0 | 39.2 | 23.4 | 37.1 | Clay loam | 6.3 | 22.3 | 25.3 | 27.5 | 29.8 | 29.6 |

* Mean of measured soil moisture contents (dried at 105$^{o}$C during 12 hrs) from 3 treatments per plots during the sampling period
# Measured at the central laboratories, IAAS, Rampur where drying was carried out at 40$^{o}$C

### 8.3.3 Determination of the density of white grubs

The densities of the white grubs were recorded at three different periods of sampling. The sampling period includes, initial sampling (1 week before fungus application), mid sampling (7 weeks after fungus application) and final (13 weeks after fungus application). The density of white grubs was determined by counting the number of individuals in four sampling holes of 0.25 $m^2$ and of depth of maximal 70 cm for each plot. At each sampling, all the insect stages (egg, larva, pupa and adults) recovered during the digging were recorded; however, collection was limited only to the maximum of thirty larvae per hole per sampling date. The same procedure was followed throughout the experiments. The larvae recovered during field sampling were placed initially in a plastic cup to get a general overview. All the living ($N_{tot)}$ and fungus killed ($N_{FK}$) individuals were counted and the unaffected live larvae were then transferred immediately into rearing vials (5 cm x 5 cm x height) filled with sterile soil. The larvae were reared with slices of potatoes in a dark room at a temperature of 22 $^oC$ throughout the rearing period. Mortality was recorded by checking the insects at weekly intervals for three months to determine the rate of *M. anisopliae* infected individuals ($IR_R$). The total infection rate ($IR_{tot}$) was calculated by the following equation (Kessler, 2004).

$$IR_{tot}\ (\%) = \frac{N_{tot}\ X\ IR_R + N_{FK}}{N_{tot} + N_{FK}}\ X\ 100$$

Where,

IR (tot) = Total infection rate
$IR_R$ = Total infection rate in the lab
N (tot) = Number of living individuals found in the field
$N_{FK}$ = Number of individuals killed by *M. anisopliae* found in the field

### 8.3.4 Application of fungus material

The fungus material was produced on peeled and sterilized barley kernels (Chapter 7) were and applied in three different dosages in the furrows made with a bullock drawn wooden plough (Figure 8.5).

The fungus kernels were applied by hand at the same place where the maize seeds were put behind the bullock drawn plough at a depth of 12-15 cm and at distance 15-20 cm. The fungus kernels were well covered by the subsequent furrow while tilling the land. The biocontrol agent (BCA) was applied in the first week of April 2005 with an objective to asses the effectiveness of *M. anisopliae* against white grubs present in the maize field. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three treatments and four replications at each site. The treatments included no application of fungus kernels ($T_1$), application of fungus kernels at the rate of 40 kg/ha ($T_2$) and application of fungus kernels at the rate of 80 kg kg/ha ($T_3$).

The observation parameter in this experiment included the assessment of *M. anisopliae* before application of the fungus containing barley kernels, 7 weeks after fungus application and 13 weeks after fungus application. At the same dates the density of white grubs and the infection rate of the white grubs due to the intervention were recorded. Further, damaged and undamaged maize roots and wilted plants were counted and crop yields determined. Soil characteristics and

weather parameters in relation to the development of the fungus were also studied in all the experimental sites.

Figure 8.5 Application of fungus in farmer's field in Nepal

### 8.3.5 Assessment of *Metarhizium anisopliae* in soil samples

The soil samples from each sampling date were assessed in order to obtain qualitative and quantitative information with regard to *M. anisopliae.* Two methods were used, the *Galleria* Bait Method (GBM) (Zimmermann, 1986) and the plating of soil suspension on selective medium (Keller *et al.* 2003). Both the methods were applied to each soil samples to isolate *M. anisopliae* and to document the presence and the density of infective propagules in the soils. The presence of *M. anisopliae* was determined with the GBM. Stones and roots were first removed from the soil; the soil was then sieved (5 mm mesh) and 60 g of each sample placed in a cylindrical plastic container (4.5 cm diameter x 6 cm height). Four late instar larvae of *G. mellonella* were added to each container. Baiting of *M. anisopliae* was performed in darkness at 22 $^{o}$C in the temperature controlled room. For the first five days the containers were inverted daily to keep the larvae moving in the soil. After 18-21 days the larvae were examined and assigned to one of the following categories: "healthy", "infected with *M. anisopliae"* and "dead from other reasons".

The density of *M. anisopliae* in the experimental plots was determined using selective medium (SM) (Keller *et al.* 2003; Strasser *et al.* 1997) and expressed as number of colony forming units (CFU) per gram of dry soil. A total of 100 g oil samples taken within each plot were mixed and sieved (5 mm mesh). Twenty grams of the homogenized sample of fresh stone- and root-free soil were added to a 200 ml Erlenmeyer flask containing 100 ml tap water supplemented with 1.8 g/l tetra-sodiumpyrophosphate ($Na_4P_2O_{7*}10H_2O$) to promote disaggregation of the soil (Fornallaz, 1992). All flasks were shaken for 3 h at 120 rpm on a longitudinal shaker. After shaking the flasks were allowed to stand for 15 S and 0.1 ml of the suspension taken from the top layer was plated on a selective medium using a Drigalsky spatula. Three replications per sample were

prepared and incubated in the temperature controlled incubator at 22 $^{o}$C. After 8-10 days the number of CFU of *M. anisopliae* was counted. To express the number of CFU g$^{-1}$ dry soil, the moisture content of the soil samples was determined by drying 100 g of fresh soil at 105 $^{o}$C for 8 h and then re-weighing.

### 8.3.6 Root damage and crop yield

In order to asses the effect of *M. anisopliae* after its application in the field root damages due to white grub attack and the differences in crop yields with respect to different treatments were recorded. These parameters were analyzed immediately after the crop harvest. The root damage caused by white grubs was assessed by pulling the ratoon and separating it from the main stalk. A total of fifteen randomly selected ratoons per plot were pulled out and their damaged roots were counted at the experimental site. The number of damaged roots was obtained out of the total plants present in a plot. In the same way, the crop yield was measured plot-wise. In order to know the final yield of maize per plot, the cobs were pulled out from each plant and made a heap nearby threshing yard. At first the cobs per plot were grossly weighed along with the husk. Afterwards, the cobs were de-husked by hand and reweighed. The difference between first weights to the second was taken as the cob yield without shelling percentage. The final grain weight after shelling from the tassel was estimated based on the following procedure used by the National Maize Research Program (NMRP), Rampur, Chitwan, Nepal.

**Procedures of obtaining maize grain yield:**

1. Weight of the cobs (with husk): This can be obtained by directly weighing the cobs with husks (kg)

2. Weight of dehusked cob (cob without husk): De-husked by hand and reweighed the cobs separately (kg)/. This can also be obtained by reducing the weight of de-husked cob from the initial weight of the cobs with husks.

3. Weight of maize grains after shelling from the maize cob: This can be obtained by multiplying with 0.8 as correction factor. This correction factor is a standard method as followed by NARC.

### 8.3.7 Studies on beetle species involved in damages

In order to know the damaging species of beetle present in the research sites, sampling was carried out in three different periods. The first sampling was carried out before application of the fungus (initial sampling), second sampling at the middle of the crop period (mid sampling) and final sampling immediately after the crop harvest. The samplings were carried out with a hand held shovel from the depth of 70 cm. All the collected specimens were reared in the laboratory and the emerged adults were identified later.

### 8.3.8 Meteorological parameters and data management

Environmental parameters such as atmospheric temperature, moisture, rainfall and relative humidity for the terai region were measured by the neighboring meteorological station of the National Maize Research Program (NMRP) of the Nepal Agricultural Research Council (NARC). The station is located in a maximal distance of 5 km from the respective experimental

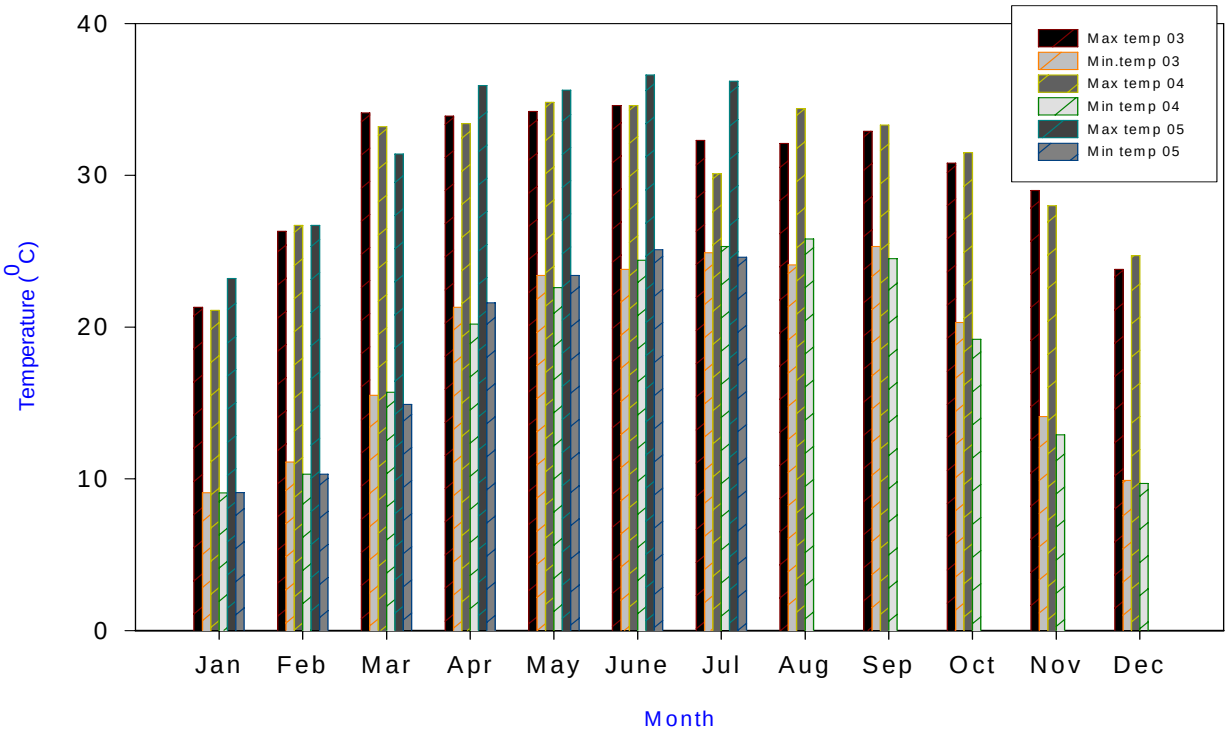
sites. At the Tanahun research site, these variables were measured from nearest meteorological station at Pokhara. At each site, the soil temperature was measured at 15 cm depth with a soil thermometer during the grub sampling period. The observations for each variable were measured three times, i.e. at the initial, mid and final samplings. Raw data obtained from the field as well as from the laboratory were entered and refined using Microsoft Excel 2000. Differences in the parameters: density of white grubs and *M. anisopliae*, infection rate, crop damage and yields were analyzed for each sampling by a one way ANOVA. Significant differences were analyzed using GENSTAT for windows 7.2 version (VSN International, Limited, Waterhouse Street, UK). Important figures (graphs) were drawn using Sigma Plot software version 6.0 (Sigma Plot 2000 for Windows, SPSS Inc., Chicago, USA).

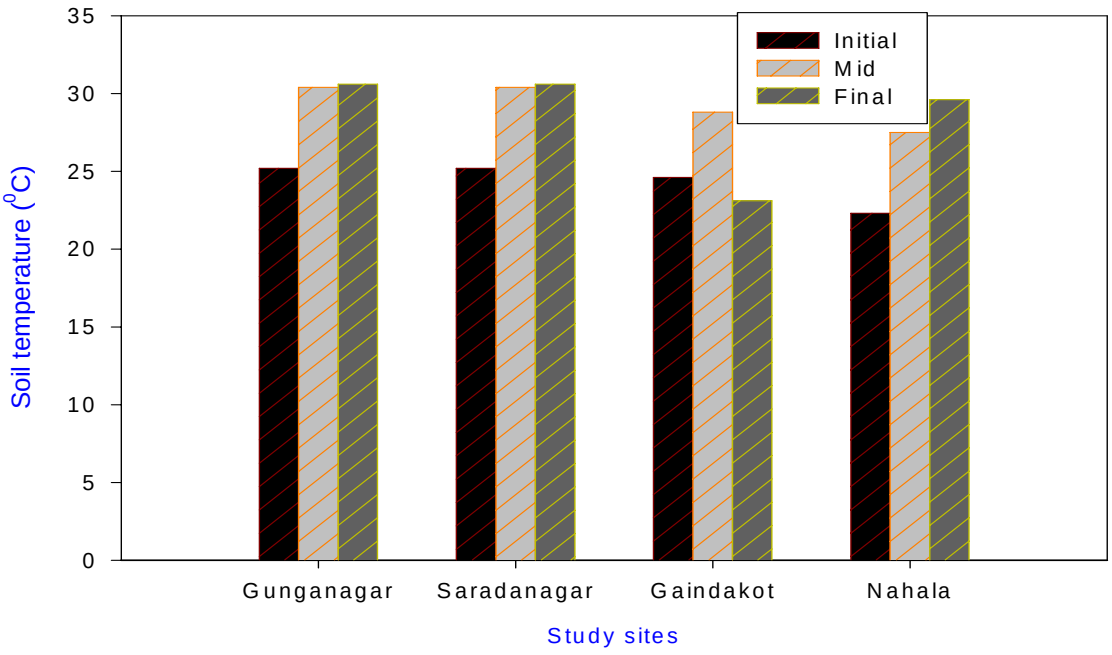
## 8.4 RESULTS

### 8.4.1 Climatic conditions in the study sites

The average atmospheric temperature measured during the sampling period from initial to final sampling in 2005 is presented in Appendix 8.3. It was found 31.4-35.6 $^{o}$C at the Gunganagar, Saradanagar and Gaindakot research sites and 25.4 -34.5 $^{o}$C at the Nahala research site. In the same way, the soil temperature in the low hill area ranged between 24.6 $^{o}$C (the lowest in March) and 32.6 $^{o}$C (the highest in June), however, the soil temperature was slightly lower (22.3 $^{o}$C-30.8 $^{o}$C) at the Nahala research site than at the former three sites. During the sampling time, the rainfall in the low hill area to mid hill remained slightly different i.e. less than 40 mm at the former three sites and more than 60 mm at the mid hill site during initial sampling. The amount of rainfall at all the sites was more than 120 mm but slightly higher at the Nahala site.

Monthly climatic data on atmospheric and soil temperature and on rainfall at the study sites are illustrated in Figures 8.6




Figures 8.6 Average atmospheric temperatures (up) soil temperatures (below) in the study sites.

### 8.4.2 Density of white grubs

The density of white grubs recovered during three different sampling periods in the experimental site is presented in Table 8.2. The study showed that the population density of white grubs at first three sites namely Gunganagar, Saradanagar and Gaindakot were not reduced ($p>0.005$) by the treatments and sampling frequencies; however, it was different at Nahala. In the first sampling i. e. before fungus application, the white grub density in Gunganagar ranged from lowest to the highest between 20-24 individuals $m^{-2}$, 11-19 in Saradanagar, 12-16 in Gaindakot and 39-50 in Nahala. At the former three sites, the white grub densities reduced considerably from the initial sampling to the mid sampling. In contrast this number increased again in the final sampling except at Nahala. During the sampling period the grub population reduced from the initial density and found as 21-45% (Gunganagar), 11.7-31.5% (Saradanagar), 50-87% (Gaindakot) and 61-70% (Nahala). It is very interesting to note that the population of white grubs in the first three sites reduced only from the initial sampling to only until the mid sampling but at the fourth site the population reduced continuously to about 75% until the final sampling. Until the mid sampling, a considerable reduction of the white grub density was found in the untreated plot but not until the final sampling. This experiment has further demonstrated that the treatment did not significantly affect the grub density, however, small differences were observed between sampling frequencies. In the experimental fields various species of grubs were found associated within the same season and in the same field which convincingly shows the involvement of large number of species (Table 8.3).

### 8.4.3 Infection rate

At most sites the infection rate with *M. anisopliae* followed the similar trend as that of white grub populations. The infection rate was higher in the treated plots (19.7 %) than untreated control plots (0 %). The treatment effect for the mid sampling was found significantly different ($p<0.005$) between the treated and untreated plots in all the sites except Gaindakot, but was not significant ($p>0.005$) for the final sampling except at the Saradanagar research site (Table 8.2). At all the study sites, the natural infection rate revealed less than 4% where it increased up to 12-15% from the time of fungus application until seven weeks after application, however, it reduced considerably in all the sites except at Nahala. At this site the infection rate with higher dose of fungus rose up to 19.7% after seven weeks, however did not increase after this time. This trend was not observed at other sites. This evidence suggests that the infection rate could have been affected by the white grub life cycle, soil moisture, temperature or other factors.

During field sampling very few specimens were found infected with *M. anisopliae,* however, others were found infected during rearing in the laboratory. The grubs collected from the fields were put into the individual vials and reared individually in the room with the same vials as collected from the field and put into observation chamber protection from the sunlight. The room temperature was controlled through the air condition (AC) at a temperature of 22 $^{o}$C throughout the recording period. The infected grubs were observed until the fungus fully sporulated on the cadavers. The identity of the fungus was confirmed with microscopic preparations.

The grubs mycosed with *M. anisopliae* are presented in Figure 8.7. Some of the suspected grubs due to fungus attacks were collected from the field and observed for infection in the lab (Figures 8.7 upper rows), whereas, some of the grubs were found as mycosed in field conditions (Figures 8.7 lower rows).

Figures 8.7 White grubs infected with *Metarhizium anisopliae* during laboratory observation after collection from fungus experiments (upper row) and succumbed in field condition (below row).

Table 8.2 Assessment of applied *Metarhizium anisopliae* at four locations* and at three different sampling period++ on white grub density, infection rate, fungus development and crop yield during the summer of 2005 (Means of four replications of four different sites).

| Study sites/ treatments* | | Density of white grubs (number/$m^2$) | | | Infection rate of white grubs (%) | | | Recovery of *M. anisopliae* as revealed by GBM** | | | Density of *M. anisopliae* (x $10^3$ $CFUg^{-1}$ dry soil) as revealed by SM*** | | | Root damage (%) | Maize yield (t/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Mid | Final | Initial | Mid | Final | Initial | Mid | Final | Initial | Mid | Final | | |
| 1 | T1 | 24 | 13 | 29 | 1.14 | 4.4a | 12.1 | 12.1 | 25.0 | 12.5 | 0.000 | 0.300a | 0.300a | 5.25b | 1.50 |
| | T2 | 20 | 16 | 24 | 2.99 | 10.7ab | 12.5 | 12.5 | 33.3 | 32.5 | 0.030 | 1.568b | 2.940b | 2.25b | 1.60 |
| | T3 | 23 | 18 | 21 | 2.53 | 19.2b | 12.5 | 12.5 | 41.7 | 32.5 | 0.033 | 1.373b | 4.475c | 1.75a | 1.58 |
| | SEM | 4.55 | 5.68 | 5.19 | 1.489 | 3.37 | 4.09 | 4.09 | 5.56 | 7.66 | 0.016 | 0.314 | 0.246 | 0.629 | 0.063 |
| | LSD (5%) | NS | NS | NS | NS | 10.78 | NS | NS | NS | NS | NS | NS | 0.789 | 2.01 | NS |
| | CV (%) | 41.1 | 72.9 | 41.9 | 134.3 | 59.1 | 66.9 | 66.9 | 33.3 | 59.3 | 0.051 | 0.058 | 0.019 | 40.8 | 8.1 |
| 2 | T1 | 11 | 12 | 28 | 1.9 | 0.0a | 8.3 | 8.3 | 8.3a | 4.172a | 0.400 | 0.172 | 0.275a | 7.25 | 1.58a |
| | T2 | 19 | 13 | 18 | 3.9 | 12.4b | 0.0 | 0.0 | 16.6a | 20.8ab | 0.138 | 1.550 | 3.725b | 5.50 | 1.68b |
| | T3 | 17 | 15 | 19 | 0.9 | 12.4b | 4.2 | 4.2 | 29.1b | 29.2b | 0.100 | 3.325 | 5.025b | 4.50 | 1.70b |
| | SEM | 5.28 | 3.34 | 5.75 | 0.264 | 3.31 | 3.68 | 3.68 | 3.71 | 5.73 | 0.209 | 0.1867 | 0.429 | 1.064 | 0.027 |
| | LSD (5%) | NS | NS | NS | NS | 10.60 | 11.76 | 11.76 | 11.86 | 18.32 | 0.669 | 0.601 | 1.374 | NS | 0.088 |
| | CV (%) | 68.8 | 48.8 | 71.5 | 160.1 | 80.1 | 176.4 | 176.4 | 41.2 | 63.4 | 0.197 | 0.022 | 0.028 | 37.0 | 3.30 |
| 3 | T1 | 16 | 2 | 8 | 3.8 | 0.00 | 4.2 | 4.2 | 8.3 | 8.3 | 0.125 | 0.080a | 0.752a | 6.25 | 4.60 |
| | T2 | 12 | 3 | 7 | 4.1 | 10.4 | 0.0 | 0.0 | 16.7 | 12.5 | 0.050 | 2.425b | 1.810b | 4.75 | 4.62 |
| | T3 | 14 | 7 | 7 | 3.8 | 12.8 | 4.2 | 4.2 | 25.0 | 25.0 | 0.550 | 4.375c | 2.495c | 3.75 | 4.68 |
| | SEM | 2.35 | 2.20 | 2.20 | 2.26 | 4.67 | 3.4 | 3.4 | 5.56 | 6.05 | 0.016 | 0.2797 | 0.205 | 0.975 | 0.124 |
| | LSD (5%) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | 0.894 | 0.658 | NS | NS |
| | CV (%) | 33.4 | 109.8 | 60.6 | 116.6 | 94.8 | 244.9 | 244.9 | 66.7 | 79.3 | 82.4 | 24.40 | 24.4 | 39.7 | 5.4 |
| 4 | T1 | 50 | 16 | 10 | 3.33b | 6.4a | 20.8 | 20.8 | 25.0 | 12.5 | 0.184 | 0.665a | 0.358a | 4.50 | 4.14 |
| | T2 | 39 | 15 | 9 | 2.50ab | 14.0b | 16.66 | 16.66 | 33.3 | 32.5 | 0.203 | 4.075b | 2.402b | 4.50 | 3.84 |
| | T3 | 40 | 12 | 10 | 0.00a | 19.7b | 16.7 | 16.7 | 41.7 | 32.5 | 0.110 | 4.575b | 2.948c | 2.50 | 4.16 |
| | SEM | 3.53 | 1.75 | 1.55 | 0.921 | 2.33 | 6.05 | 6.05 | 5.56 | 7.66 | 0.054 | 0.2469 | 0.161 | 1.167 | 0.207 |
| | LSD (5%) | NS | NS | NS | 2.948 | 7.45 | NS | NS | 17.78 | NS | NS | 0.7897 | 0.516 | NS | NS |
| | CV (%) | 16.5 | 24.8 | 32.4 | 94.8 | 34.2 | 67.1 | 67.1 | 33.3 | 59.3 | 65.3 | 15.9 | 17.0 | 60.9 | 10.2 |

* Study sites: 1 = Gunganagar, Chitwan; 2 = Saradanagar, Chitwan; 3 = Gaindakot, Nawalparasi; 4 = Nahala, Tanahun, ** GBM = *Galleria* Bait Method; *** SM = Selective Medium T1, T2, T3 = treatment with a dose of 0: 40 and 80 kg/ha dosages for 1, 2 and 3 respectively; SEM = Standard error of mean; LSD = Least significant differences CV = Coefficient of variation + Figures in column followed by same letter are not significantly different at p<0.010 by DMRT

++ Assessment period = initial = before fungus application; mid = after 7 weeks after fungus application and during crop duration and final = immediately after crop harvest i. e. after 13 weeks of fungus application

### 8.4.4 Recovery of *M. anisopliae* with the *Galleria* bait method (GBM)

The trial sites were treated in the spring of 2005 with the indigenous BCA produced at the Insect Pathology laboratory of IAAS, Rampur. The presence of naturally occurring *M. anisopliae* in the soil was determined before the application of the BCA. After the application, the development of the fungus was monitored from the soil samples taken after three and six months using the GBM and with soil suspensions spread on selective medium (SM). The frequency of infected *Galleria* larvae from soil samples of Gunganagar ranged between 25-41.7% after seven weeks of fungus application and between 12-32.5 % after thirteen weeks. No significant difference was observed between the treated and the untreated plots. In the same way, the frequency of infected *Galleria* larvae from soil samples taken at Gaindakot and Nahala did not change significantly over the time except for the mid sampling at the Nahala research site (Table 8.2). At these sites the frequency of infected larvae ranged from 0-41.7%. In contrast to these sites, the frequency of infected *Galleria* larvae at the Saradanagar research site differed significantly ($p<0.005$), where the density rose considerably both at the mid and the final sampling with 8.3-29.1% and 4.17-29.2% respectively.

### 8.4.5 Density of *Metarhizium anisopliae* determined with selective medium

The soil samples which were analysed with the GBM were also analysed with soil suspensions spread on selective medium (Figures 8.8-8.9).


Figure 8.8 Soil plating for the determination of fungus density from soil samples (Soil suspension method)

Figure 8.9 Fungus densities into the selective medium after isolation from the soils of experimental plots

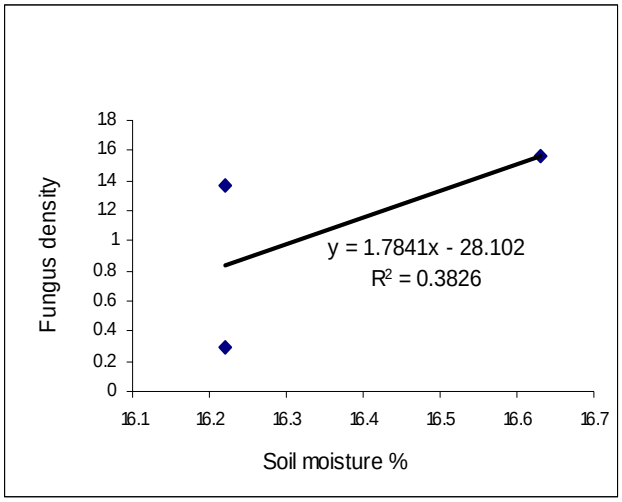
BCA application at Gunganagar resulted in a maximal density of *M. anisopliae* 4.475 x $10^{3}$ CFU $g^{-1}$ dry soils (Table 8.2). The density of *M. anisopliae* from soil samples from this application revealed a significant increase ($p<0.005$) from the mid to the final sampling. A similar trend of increase in density was observed at Saradanagar with 0.10-5.02 x $10^{3}$ CFU $g^{-1}$ dry soils when observed before fungus application and after 13 weeks. At this site, the density of the fungus changes among the treatments over the time. The density of fungus as indicated by the number of CFU $g^{-1}$ dry soil at Gaindakot and Nahala increased significantly ($p<0.005$) until the mid sampling, however, decreased eventually at the final sampling. The density of the fungus at these sites ranged between 0.05- 4.457 CFU $g^{-1}$ dry soils however, no significant increase of the fungal density was detected within the sampling frequencies.

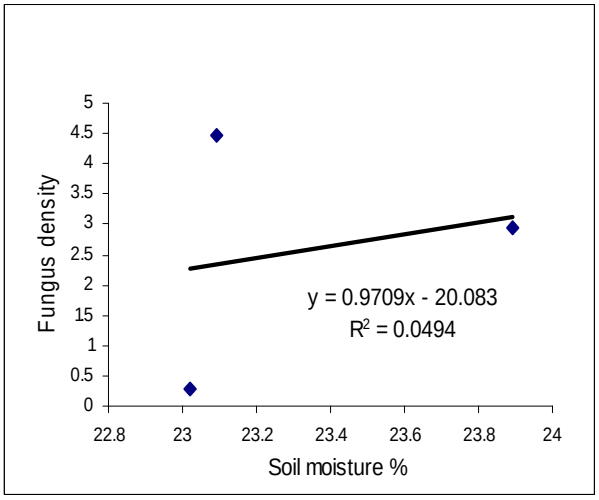
We measured significant increases of the fungus densities between the treatments. It is interesting to note that the number of CFU of *M. anisopliae* in the control plots of all the sites did not change significantly over the sampling duration. The changes of the fungal density at Nahala showed an increasing trend from initial to final and vice versa to the grubs' density.

### 8.4.6 Influence of soils properties on the establishment of *M. anisopliae*

The BCA applications were conducted in soils of different soil texture, pH, moisture and temperature (Table 8.1). The overall characteristics of the soil at three terai areas were sandy loam whereas it was clay loam at the Nahala site. Despite the differences in the soil texture, there was not much variation in the pH at all the study sites where it ranged from 5.50 - 6.35. No apparent relation in terms of white grub population and establishment of fungus density could be observed due to soil characteristics.

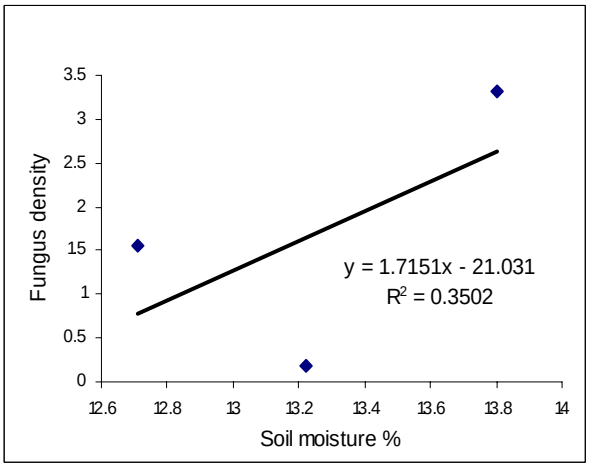
At these sites, the soil temperature over the sampling period of the sampling ranged between 25-31 $^{o}$C at low hill sites, it was slightly lower (22.3-29.8 $^{o}$C ) at the mid hill site. Similarly, the soil moisture at the study site was between 15-25% at the low hill sites and 18-26% at the mid hill site from initial to final sampling. The influence on the establishment of *M. anisopliae* due to soil moisture at seven and thirteen weeks after the application showed a positive correlation (r = 0.38 and r = 0.049; p< 0.005) respectively in Gunganagar and (r =0.350 and r =0.957; p<0.005) Saradanagar research sites (Figures 8.10-8.13).

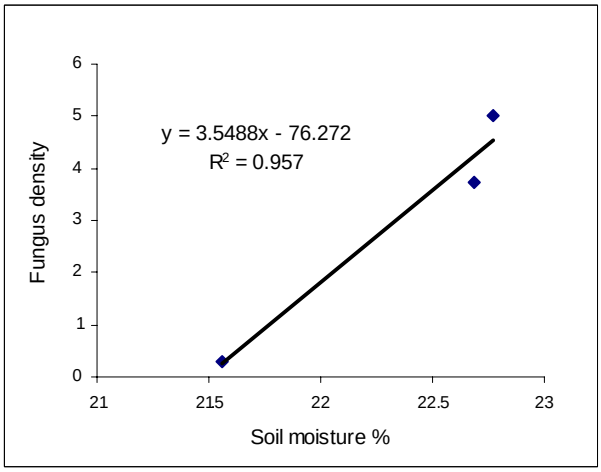



Figures 8.10-8.11 Development of the density of the BCA seven (left) and thirteen (right) weeks after the application with respect to soil moisture in Gunganagar, 2005.

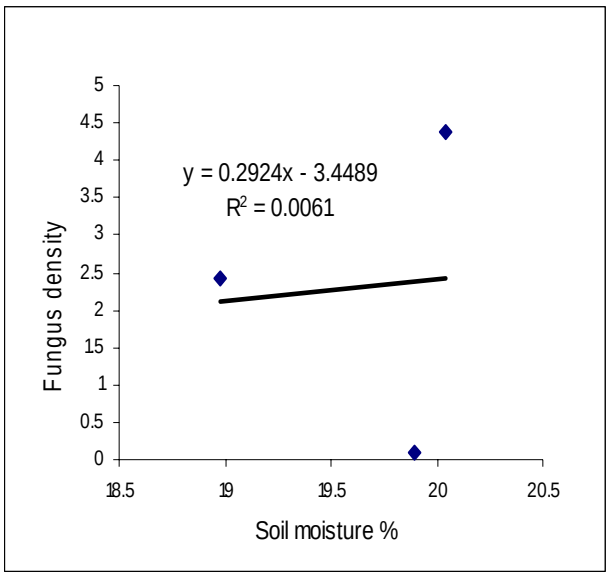
The correlation study has confirmed that, the number of CFU significantly increased from 0.000 in initial sampling to 5.025 until 13 weeks in sandy soils (sites 1-3) however, we observed decreased fungus densities in site 4 from the 4.575 during 7 weeks to 2.948 until 13 weeks in the clay soils (Table 8.2).

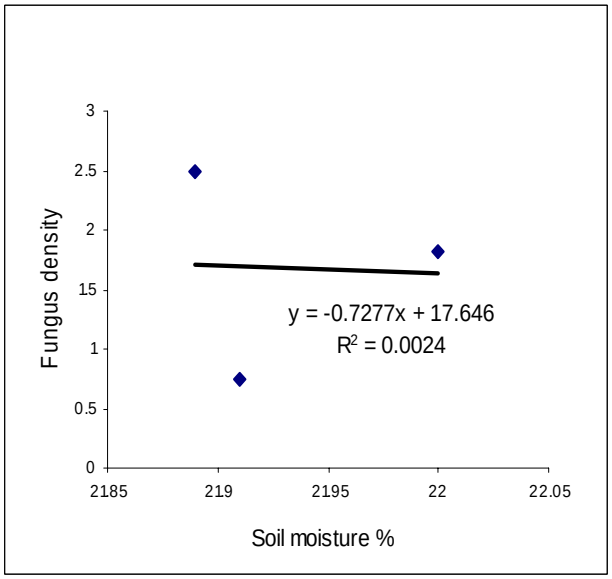


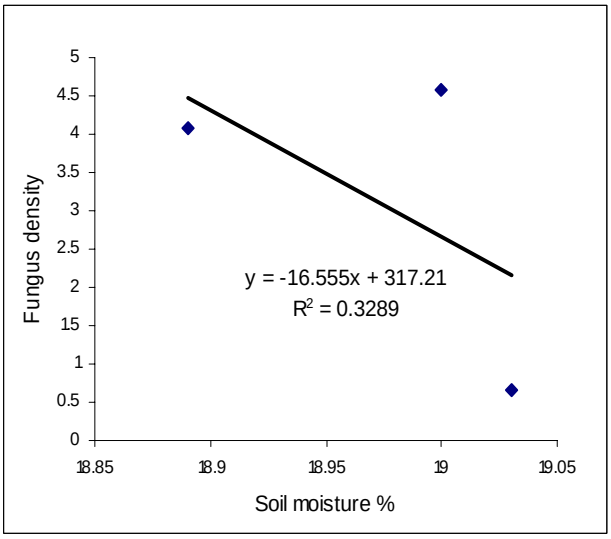

Figures 8.12-8.13 Development of the density of the BCA seven (left) and thirteen (right) weeks after the application with respect to soil moisture in Saradanagar, 2005.

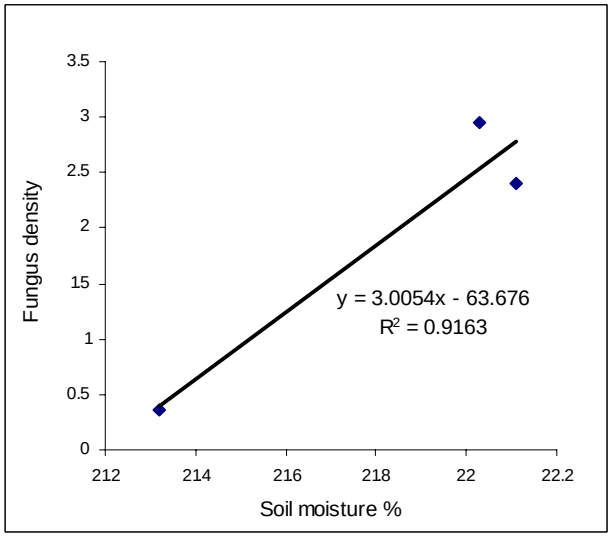
In contrast to these sites the establishment of *M. anisopliae* was very slow at Gaindakot and even negatively correlated at Nahala in 7 weeks however, but was positively correlated after 13 weeks (Figures 8.14-8.17).



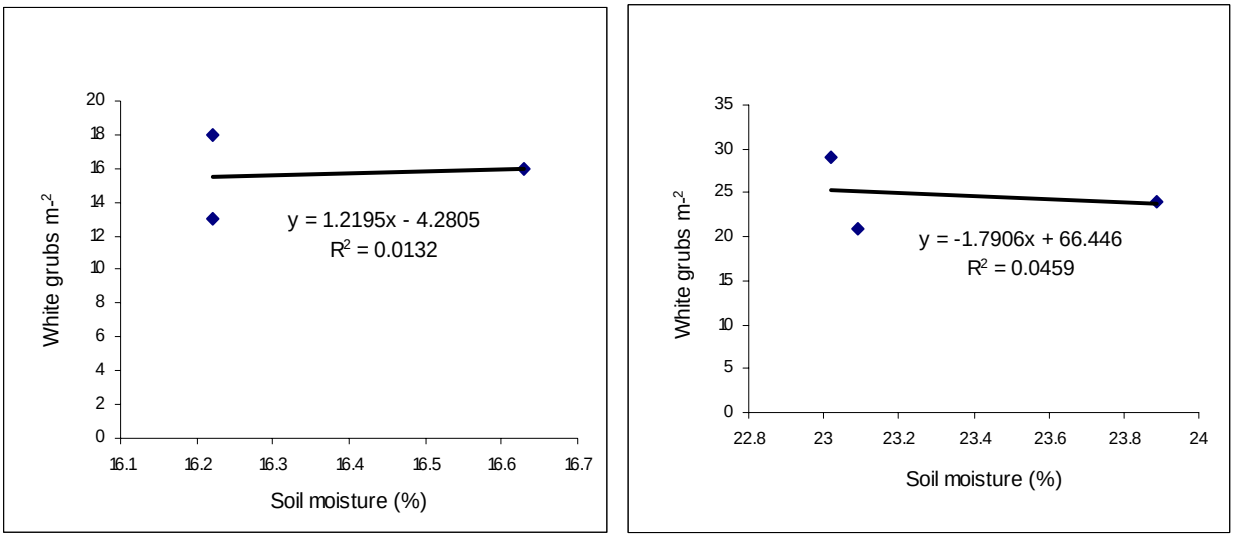

Figures 8.14-8.15 Development of the density of the BCA seven (left) and thirteen (right) weeks after the application with respect to soil moisture in Gaindakot, 2005.




Figures 8.16-8.17 Development of the density of the BCA seven (left) and thirteen (right) weeks after the application with respect to soil moisture in Nahala, 2005.

Unlike the fungus density after the application in the experimental plots, the white grub density was found negatively correlated with increasing soil moisture at the Gunganagar and Saradanagar research sites respectively (Figures 8.18-8.21).




Figures 8.18-8.19 Density of white grubs at seven (left) and thirteen (right) weeks of sampling with respect to soil moisture in Gunganagar, 2005.

In Nahala research site, the grub density reduced sharply with increasing soil moisture (Tables 8.1 and 8.2). Therefore, the grub population was not only dependent from soil moisture. Probably other factors such as the insect biology and host crops, soil and biotic factors might have significant influence.

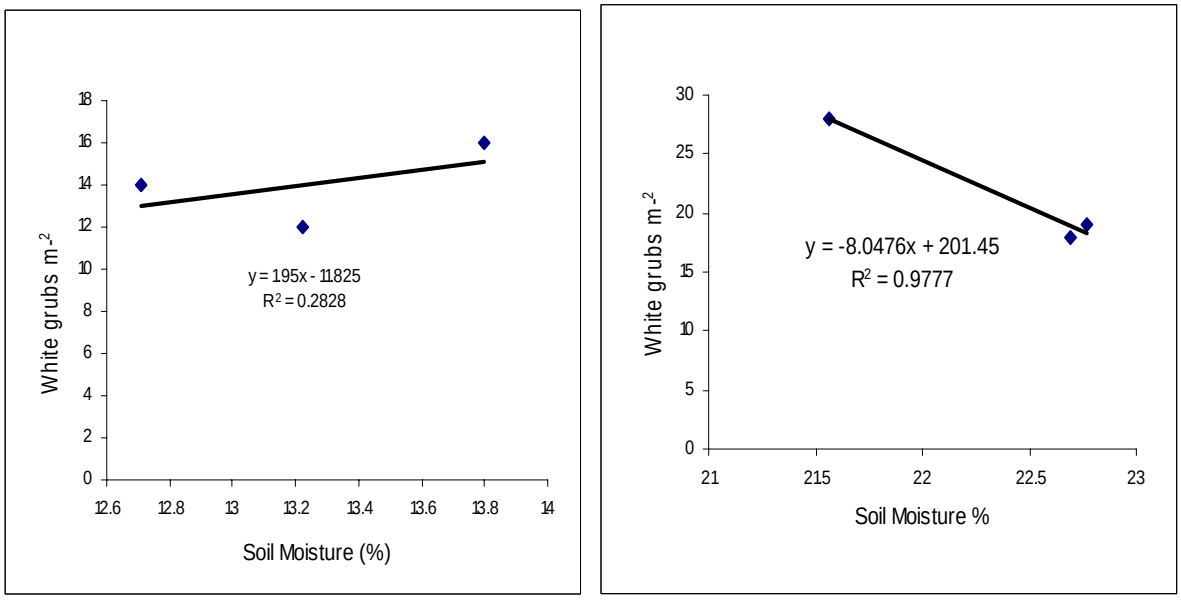



Figure 8.20-8.21 Density of white grubs at seven (left) and thirteen (right) weeks of sampling with respect to soil moisture in Saradanagar, 2005.

White grub density with respect to soil moisture was also not significantly correlated at the Gaindakot and Nahala research sites, it remained rather constant in some cases (Figures 8.22-8.25) and slightly increased until the mid sampling at Gaindakot.

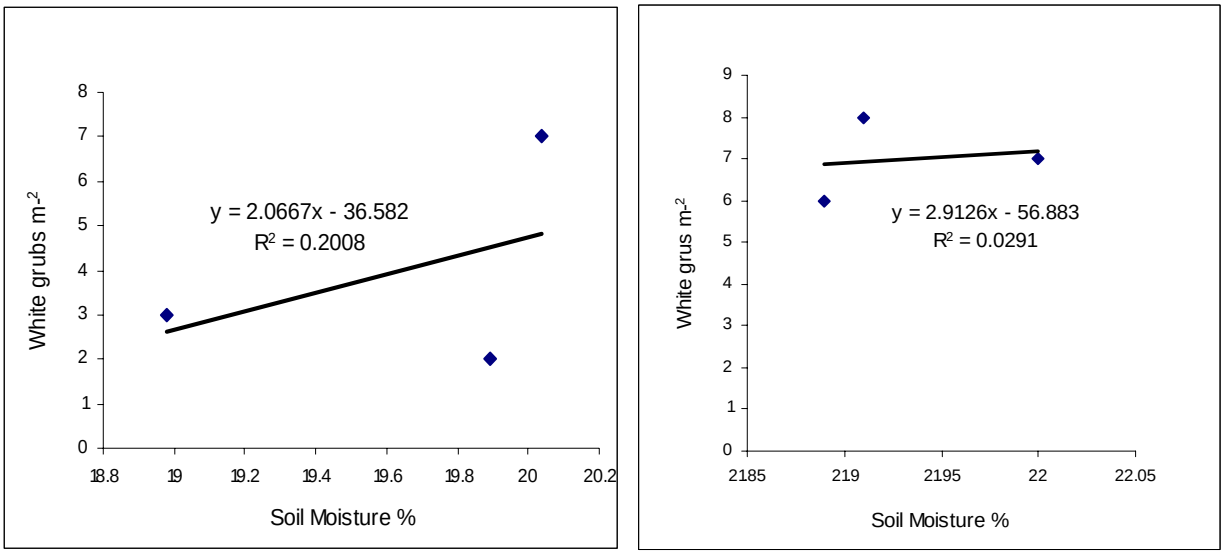



Figure 8.22-8.23 Density of white grubs at seven (left) and thirteen (right) weeks after samplings with respect to soil moisture in Gaindakot, 2005.

Unlike at the previous three study sites, the white grub density at Nahala, a mid hill research site, remained positively correlated at the final sampling. The possible reason might be due to the presence of different stages of beetle species. This is in contrast to the former sites where different beetle species with overlapping generations are common; however, in Nahala research site, *Phyllognathus dionysius* found one of the dominant species. Several grub species were involved at the harvesting stage of the crops in former sites where the moisture level at that time was also high.

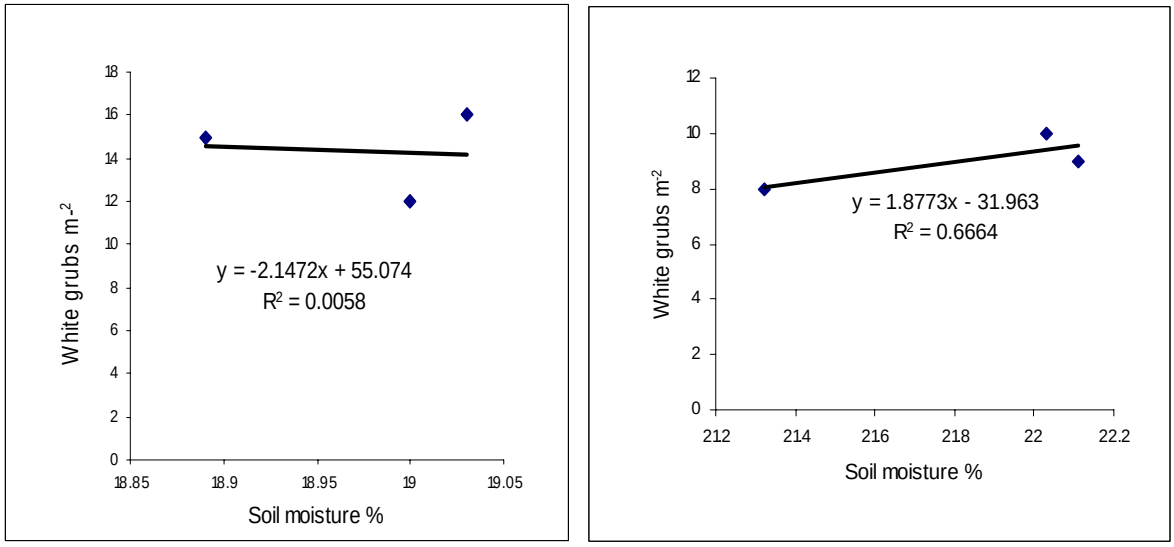



Figure 8.24-8.25 Density of white grubs at seven (left) and thirteen (right) weeks after samplings with respect to soil moisture in Nahala, 2005.

This experiment has revealed that the density of white grubs in all the study sites decreased from initial to mid sampling, however, this trend did not continue until the final sampling. In the same way, the fungus density did not differ more in the final sampling from that of the mid sampling, as the density remained in the similar trend.

### 8.4.7 Root damage and crop yield

These two parameters were assessed in order to determine the effect of *M. anisopliae* on damage and yield. The study demonstrated that the treatment had no significant effect on root damage at all the study sites except Gunganagar ($p<0.005$). At this site, the root damage ranged between 1.75 -5.25%.

Similarly, the effect of applied BCA on crop yield showed the similar trend as found for the root damage; the maize grain yield did not differ significantly ($p>5\%$) except at the Saradanagar research site. In general the crop yield in all the research sites was found fairly low than the national production and productivity. The possible reason for this might be due to untimely rainfall during maize sowing time as maize farming in Nepal depends on seasonal monsoon.

This study further revealed that white grubs congregate (Figure 8.27, lower right) at the secondary and even tertiary root portion when the crop matures.

Figures 8.26 Harvesting (left) and transportation of maize (right)

The damaged root of maize showed the association of grubs even until harvesting time of the maize. Based on this information, it can be concluded that, white grubs are available round the year in such fields and the grubs are damaging species. This result has clearly revealed that many species of Scarab beetles are involved in the maize fields of the experimental sites. Very interestingly nearly a dozen species are dominant at low hill terai research sites compared to mid hills.

Figure 8.27 Assessment of maize yields (left) and roots affected by white grubs (right) in the field

### 8.4.8 Studies on beetle species involved in damages

The beetle species that were commonly present at the fungus application sites are presented in Table 8.3.

Table 8.3 White grubs species recovered at four different research sites in Nepal in the fungus application experiments in 2005 (value in the parentheses indicate the number of beetles (collected per $m^2$).

| Collection site | Date of collection | Sampling frequency | Beetle species |
|---|---|---|---|
| Gunganagar, Chitwan (230 m, 27°39' N, 84°19 E) | 15.03.2005 | Initial | *Schizonychia fuscescens* Blanchard (1), *Maladera affinis* Blanchard (1), *Adoretus versutus* Harold (2) |
| | 15.05.2005 | Mid | *Allisonotum simile* Arrow (2), *Heteronychus* sp. 2 (1) *Apogonia* sp. 1 (1), *Anomala cantori* Hope (1) |
| | 19.06.2005 | Final | *Holotrichia seticollis* Moser (4) |
| Saradanagar, Chitwan (230 m, 27°38.5' N, 84°22 E) | 18.03.2005 | Initial | *Maladera affinis* Blanchard (2), *Anomala dimidiata* Hope (2) |
| | 3.05.2005 | Mid | *Heteronychus lioderes* (2), *Heteronychus* sp. 2 (1) *Adoretus lasiopygus* Burmeister (1), *Adoretus versutus* Harold (1) |
| | 28.06.2005 | Final | *Holotrichia seticollis* Moser (3), *Idionychus excisa* Arrow (1) *Anomala bilobata* Arrow (1) |
| Gaindakot, Nawalparasi (205 m, 27°42.5' N, 84°25 E) | 30.03.2005 | Initial | *Idionychus excise* Arrow (1), *Maladera affinis* Blanchard (1) *Anomala bilobata* Arrow (1), *Anomala xanthoptera* Blanchard (1), *Mimela siliguria* Arrow (1) |
| | 4.06.2005 | Mid | *Maladera affinis* Blanchard (1), *Sophrops* spec. 5 (1) *Adoretus versutus* Harold (1), *Anomala varicolor* Gyllenhal (3) |
| | 3.07.2005 | Final | *Chiloloba acuta Wiedemann* (1), *Phyllognathus* |

| | | | |
|---|---|---|---|
| | | | *dionysius* F (1), *Holotrichia seticollis* Moser (1), *Anomala variegata* Hope (2) |
| Nahala, Tanahun (950 m, 28°11.5' N, 83°21 E) | 30.03.2005 | Initial | *Oxycetonia sp.* (1), *Phyllognathus dionysius* F. (2) |
| | 4.06.2005 | Mid | *Phyllognathus dionysius* F. (3) |
| | 3.07.2005 | Final | *Phyllognathus dionysius* F. (2) |

In this site, *Phyllognathus dionysius* was commonly found. No definite trend in the occurrence of the beetle species was followed across the sampling period, however, some of the beetle species such as *Maladera, Anomala* and *Adoretus* are common during initial to mid sampling i.e. March-April through June-July, whereas *Holotrichia* appeared later in the crop i.e. around harvesting time and this is common in all low land sites.

## 8.5 DISCUSSION

The establishment of *M. anisopliae* after its application at different sites of Nepal is discussed. The aim was to investigate the establishment of BCA in different soil types and the influence on root damage crop yield.

The changes in the density of white grubs and in the infection rates were monitored with different samplings. Similarly, changes in the density of *M. anisopliae* were assessed using two different methods, the *Galleria* bait method (GBM) and by counting colony forming units (CFU) on a selective medium. Significant changes in the frequency of fungus infections and in the fungus density were found using with these methods. Comparison of both methods suggests that GBM gives qualitative information on presence or absence of the fungus in the soil whereas the second method provides quantitative information i.e. the density of fungus propagules in the soil. In other words, the former method is robust and simple; the later demands good equipments and techniques of assessment, however, this method provides more information on virulence as reported by Kessler (2004). He reported *B. brongniartii* could be re-isolated after sixteen months as virulent through GBM. Different factors may affect the assessment process of establishment and survival of the fungus.

### 8.5.1 White grub densities

At every sampling, there was a large difference in the density of white grubs among the research sites. At the Nahala research site, almost three times more grub was recorded as compared to the research sites of Gunganagar, Saradanagar and Gaindakot. The possible reason behind this might be due to the differences in altitude, soil characteristics, aspect of the research sites, vegetation and largely due to the species composition and their different biology. Since Nahala is located in the mid hill area and the other three sites in the terai belts most of the environmental parameters are also different. More importantly the white grub species present at these sites is different. At the low altitude research sites, mostly tropical white grub species with short life cycles such s *Maladera, Anomala, Allisonotum* and *Heteronychus* are more common whereas in mid altitude, *P. dionysius* is the dominant species along with other species such as *Holotrichia* (Table 8.3). These species have different nature of association with the vegetation and host crop which might have significantly influenced their presence and absence during different periods of sampling. At the same time, little effect of the treatment was noticed on the populations of white grubs until

three months after fungus application. However, this effect was not significant until the final sampling indicating the need of further studies considering different seasons of application and assessment. Similarly, it is necessary to study the post effect and the persistence of the fungus once applied in the soil to draw the valid conclusion about the treatment effect.

### 8.5.2 Infection rate

During field sampling none of the grubs were found infected due to the naturally occurring *M. anisopliae*. Before the application of *M. anisopliae,* very low numbers of grubs were found infected during the laboratory recording. This finding suggests the very low density of naturally occurring fungus in the experimental plots indicating the need for further augmentation. Following the application of *M. anisopliae* increased infection rates were observed until three months; however, the infection rates decreased after this time. Kessler (2004) reported that there is generally an average increase of $1.5 \times 10^3$ CFU $g^{-1}$ dry soil three months after application of the "*Beauveria*-Schweizer" product (40 kg $ha^{-1}$). The extent of fungus growth in our experiment from the seven weeks (mid sampling) to the thirteen weeks (final sampling) increased in the same manner, however the low infection rates after crop harvest may be associated with the shorter persistence of the fungus in the soils coupled with low density of white grubs and increasing soil temperatures. In general, these factors did not result in any significant infection rates and will be a subject of future experimentation.

### 8.5.3 Establishment of *M. anisopliae* in the soil

The establishment of the fungus in the soil was demonstrated by both methods of analysis such as through the *Galleria* bait method and the selective medium method. In most cases there was a significant increase in the number of CFUs of *M. anisopliae* in the soil seven weeks after the application of the BCA. In contrast, the fungus density did not increase continuously in some of the sites until thirteen weeks after the application. Between two sampling intervals from mid to the final, the fungus densities increased at the Gunganagar and Saradanagar research sites, however, reduced at Gaindakot and Nahala. This finding suggests a strong influence of climatic factors such as soil properties, soil temperature, moisture and humidity on the growth and establishment of the fungus. The possible reasons for this are also linked with the host density which was reduced at Gunganagar and Saradanagar in contrast to the former sites (Table 8.2). Similar pattern of reduction of the fungus density due to host factors is reported for *B. bassiana* (Zimmermann, 1986; Gaugler *et al.* 1989) and *Nomuraea rileyi* (Ignoffo *et al.* 1978). The survival of entomopathogenic fungi in the soil is mainly attributed to abiotic factors, such as temperature, humidity and other soil characteristics, and to biotic factors such as antagonistic microorganisms and metabolites of soil organisms. The later factors, although important, could not been assessed in this study and no definite correlation was revealed between the survival of *M. anisopliae* and data on soil properties (texture, moisture, pH, temperature). However, small differences were recorded at the study sites.

Among several biotic factors, biodegradation is another factor potentially involved in the decline of the abundance of fungi in the soil (Fargues *et al.* 1983). Such activity is reflected in a high microbiological activity and high content of organic matter in the soil; however, we could not analyse the effect of biodegradation and fertility status in the soil which should be considered in future studies. Similarly, clay particles in the soils are reported to prolong the persistence of fungal spores in the field (Amir and Alabouvette, 1993) and have been used as a protectant

against biodegradation (Keller and Zimmermann, 1989). However, in our present study no correlation was found between the extents of reduction of *M. anisopliae* and the clay content in the soils (Table 8.1).

### 8.5.4 Influence of soil temperature

Temperature is known to be one of the most important factors influencing the development of an organism. Goettel and Inglis (1997) reviewed the effect of temperature extensively. Most of the entomopathogenic fungi have a wide range of temperature tolerances (0-40 $^{o}$C), however, temperature optima for infection, growth and sporulation are usually much more restricted (generally 20-30 $^{o}$C). Propagules for most species survive well at sub-zero temperatures and can be stored for long periods at -20 $^{o}$C or in liquid nitrogen (-196 $^{o}$C). Spores of some species can tolerate very high temperatures for very short periods (150 $^{o}$C for 30 seconds); however, the maximum threshold for long periods is usually close to 40 $^{o}$C (Goettel *et al.* 2000). On Sabouraud-destrose-agar as well as on barley kernels, *M. anisopliae* is able to vegetatively grow at temperatures between 2 and 33 $^{o}$C, to germinate between 2 and 27 $^{o}$C and sporulated between 5 and 28 $^{o}$C. The optimum temperature for *M. anisopliae* infecting adult thrips is 23 $^{o}$C (Vestergaard *et al.* 2002), and a decrease in temperature of 3-5 $^{o}$C increases the time to death by 1 day. In general, the optimum temperature for most entomopathogenic hyphomycetes is between 20 and 25 $^{o}$C but infection and disease can occur at temperatures ranging between 15 and 30 $^{o}$C. Above 30 $^{o}$C, the vegetative growth of most taxa is inhibited and growth usually ceases at 37 $^{o}$C.

Our field experiments indicated a correlation between the growth of the fungus until seven weeks from the time of application at which the soil temperature and moisture were not more than 25 $^{o}$C and 18% respectively (Table 8.1). This experiment has further indicated the lack of optimal soil temperature especially when the fungus application was carried out during the summer season. It is because optimal soil temperature were not achieved during the experimentation, the temperature was not conducive especially onwards May-June when the fungus development started. In most part of the terai areas of Nepal, maize is also sown in spring season and white grub problems are also reported during this period. Looking into the soil temperature for the establishment of BCA, application may be carried out during this season. Cool temperatures in the late autumn and early spring probably inhibit the initial development of *M. anisopliae;* however, such months ceases soon and temperature starts to become conducive as soon as the spring finishes. An effect of soil temperature on colony forming units of *B. bassiana* on *Spodoptera* pupal cadavers is also reported by Studdert and Kaya (1990).

The difference in CFUs recovered from different soils of the research sites located in terai and in the mid hill area also suggests the effect of soil properties on the establishment and survival of the BCA. The soil properties at the low hill terai research sites were fairly sandy but clay loam at the mid hill research site. This could be one of the possible reasons for the differences in the density of the fungus between the treated and the untreated plots. The possible hypothesis may be the low binding capacity of the sandy soils for fungus spores as compared to clay soils; however, such aspects were not covered in these experiments which could be a subject of future studies. Furthermore, the treated area at terai research sites were more exposed to the sunlight unlike to the site in the mid hill where it was a comparatively shaded area surrounded by fodder trees.

### 8.5.5 Influence of the relative humidity and soil moisture

Soil moisture is another important factor affecting the development of microorganisms. Most entomopathogenic fungi need 95-100% relative humidity (RH) for optimal development (Luz and Fargues, 1997 and 1998; Hallsworth and Magan, 1999). As RH values in the soil range around 99%, conditions are generally suitable for fungal development (Griffin, 1963). Generally, many literatures suggest humidity conditions should be favorable for fungal growth, however, in our experimental area, it was very dry at the time of fungus application with a water content of 71.2-98%. This could probably be one the possible reasons for the poor establishment of the fungus in the soil. Another variable that might influence fungal growth is the water content of the soils. However, the apparent differences in the establishment of the fungus due to soil moisture could not been observed except at Gunganagar and Saradanagar. Similarly, the rainfall at the study sites was not much different throughout the study and consequently an effect of rainfall on the fungus density could not be detected.

From these results it can be concluded the soil moisture and rainfall at the study sites had no profound effect. In fact, water content has been reported to have no direct effect on fungal growth (Griffin, 1969) and no major influence on growth and sporulation of the fungus. During the experimentation, no heavy rainfall was recorded across the site which further suggests no any detectable clues on the insect infection and density of the fungus. Fungus application in Gunganagar and Saradanagar research sites was carried out in March where 38.9 mm rainfall was recorded in these sites. Unlike these two sites, fungus application in Gaindakot and Nahala research sites were conducted in the month of May where the initial rainfall was 43.1 mm and 65.1 mm respectively. In the same way, amount of rainfall at the harvesting time in Gunganagar and Saradanagar research sites was recorded 165.3 mm in the month of June. However, in Gaindakot and Nahala maize crop was harvested in July and the rainfall in these sites was 155.3 mm and 185 mm respectively.

### 8.5.6 Influence of soil pH

Soils with extreme pH values are known to be highly suppressive to several plant diseases, whereas soil microbes are only slightly affected by intermediate pH values (Alabouvette *et al.* 1992; Griffin, 1994). As the pH values in our study sites were almost at the same scale between 5.50 – 6.35 the growth of *M. anisopliae* was not expected to be adversely affected. In our experiment, no correlation was found between soil pH and growth of *M. anisopliae*. Incase of any influence of this parameter for the growth and establishment of the fungus would be one of the important investigations in the future study.

### 8.5.7 Root damage and crop yield

The effect of the white grubs on root damages and crop yields was studied only after harvesting of the crop. No significant differences were observed for both of the parameters. Despite of the insignificant differences for observable damages to maize yields, the grubs were found associated in the maize roots even after crop harvest. From this study it is realized that some visible differences could be noticed if these measurement could have been conducted at earlier stages of the crop and at least at every sampling time rather than a single recording at the final stage of the crop. Future assessment for root damage assessment should be carried out at every sampling.

### 8.5.8 Studies on beetle species involved in damages

The possible reason of the sparse distribution of many scarab species at the low hill site may be due to the cultivation of the ranges of crops coupled with tropical temperature. At these sites, many overlapping generations are common which probably might have favored by the round year availability of the crops. In contrast to these sites, the cropping system is different in mid hill sites, Nahala, Tanahun where farmers relay maize crops with upland rice and land remains fallow for some period (at least more than a month). This pattern of agriculture might have favored for the regular occurrences of *P. dionysius* in this site. This beetle was found as one of the dominant species at this site which was also revealed by light trap studies (Chapter 9). In other words, these beetle species are major composition of this site. The community people put their opinion for the regular occurrences of this beetle species, which probably might have favored by well protected sallo (*Pinus* spp*)* and fodder trees such as khanyu (*Ficus* spp) into their farmland.

## 8.6 CONCLUSIONS

The establishment of a high density of *M. anisopliae* in the soil can be achieved by applying the BCA with higher dose if it is applied only once in the crop duration. However, the modest dose may be applied splitting twice or three times during a crop season. The barley kernels should also be fully colonized by the spores of *M. anisopliae.* It is always recommended to check the quality before the application. A satisfying quality is achieved when more than 90% of barley grains are colonized with pure *M. anisopliae.* At the same time, higher infections may be achieved when the applications are coincided at a period when conditions for growth and sporulation are suitable. Among many factors, soil texture and temperature were shown to be the most crucial factors. The influence of soil type appears to play only a secondary role in the successful establishment of the fungus. At the same time biotic factors coupled with different host species with different life span and life cycles are probably of greater importance than soil texture. After the initiation of an epizootic in the host populations, the survival of the fungus is mostly dependent on host density (Chapter 11). Therefore, success and failure of a biological control of white grubs with *M. anisopliae* strongly depends on the production and application of sufficient infectious material in the soil considering biotic and abiotic factors. Moreover, additional research is needed to determine what factors are presently limiting the establishment and infection of *Metarhizium* in the field. Similarly, identification of damaging species and understanding of their biology are other important aspects for the success of these control method.

In Nepal, the use of microbial pesticides is one of the new approaches; however; *M. anisopliae* has been commercially used in a number of other countries where health and environmental considerations are important. In addition, the products are very useful against those pests that are common in the soil and other cryptic habitats. They can be specifically targeted mainly to those pests that are difficult to control with commonly available chemical pesticides because of their difficult biology. Many pests have already developed resistance against chemicals and some of these compounds are largely blamed as a source of environmental pollution which also causes human health hazards. On this background production and use of *M. anisopliae* with indigenous strains would be one of the corner stone for organic pest management in the country. The indigenous origin of these strains satisfies the requirements for proceeding ahead for microbial products. In case of Nepal the situation is very favorable since no chemical pesticide

manufacturing industry exists in the country. The use of biological products is largely praised by the farmers, consumers, government agencies and donor communities. Similarly, the application of such products would be cost effective in the long run when production methods are adapted to the local situation even though it seems costlier in the beginning. Therefore, coordinated efforts in production and application at multilocation experiment should be carried out before promoting them into wider scale to the farmers. Eventually, production and distribution should be carried out by the private organizations with due support of the subject matter specialists.

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**Appendices**

**Appendix 8.1 Monthly averages of climatic parameters of Gunganagar, Saradanagar (Chitwan) and Gaindakot (Nawalparasi), Nepal, in 2003. (Source: MRP, Rampur)**

| Recording month | Atmospheric temperature ($^{o}$C) | | Soil temperature ($^{o}$C) | | Relative humidity (%) | Rainfall (mm) |
|---|---|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum | | |
| January | 21.3 | 9.1 | 19.3 | 13.1 | 98.2 | 42.4 |
| February | 26.3 | 11.1 | 22.6 | 14.3 | 96.9 | 54.0 |
| March | 34.1 | 15.5 | 26.4 | 21.8 | 84 | 85.0 |
| April | 33.9 | 21.3 | 31.7 | 21.6 | 74.2 | 120.6 |
| May | 34.2 | 23.4 | 32.9 | 26.2 | 78.0 | 132.2 |
| June | 34.6 | 23.8 | 32.6 | 29.8 | 78.2 | 135.6 |
| July | 32.3 | 24.9 | 32.8 | 28.9 | 87.3 | 201.5 |
| August | 32.1 | 24.1 | 31.3 | 32.4 | 84.7 | 264.1 |
| September | 32.9 | 25.3 | 31.9 | 27.6 | 88.9 | 227.3 |
| October | 30.8 | 20.3 | 29.5 | 23.2 | 86.1 | 76.2 |
| November | 29.0 | 14.1 | 24.2 | 17.3 | 92.5 | 31.0 |
| December | 23.8 | 9.9 | 20.6 | 16.5 | 98.0 | 0.0 |

**Appendix 8.2 The monthly averages of climatic parameters at Gunganagar, Saradanagar (Chitwan) and Gaindakot (Nawalparasi) Districts, Nepal, in 2004. (Source: HMRP, Rampur)**

| Recording month | Atmospheric temperature ($^{o}$C) | | Soil temperature ($^{o}$C) | | Relative humidity (%) | Rainfall (mm) |
|---|---|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum | | |
| January | 21.1 | 9.7 | 21.3 | 13.8 | 98.3 | 62.7 |
| February | 26.7 | 10.3 | 21.7 | 15.2 | 97.9 | 0.0 |
| March | 33.2 | 15.7 | 27.9 | 21.2 | 82 | 0.0 |
| April | 33.4 | 20.2 | 30.6 | 24.8 | 75.3 | 180.2 |
| May | 34.8 | 22.6 | 33.3 | 27.8 | 75.06 | 111.4 |
| June | 34.6 | 24.4 | 33.2 | 29 | 79.8 | 15.8 |
| July | 30.1 | 25.3 | 31.9 | 29.2 | 88 | 15.5 |
| August | 34.4 | 25.8 | 33.4 | 30.2 | 85.6 | 214.3 |
| September | 33.3 | 24.5 | 32.7 | 28.7 | 87.5 | 417.7 |
| October | 31.5 | 19.2 | 28.6 | 24.5 | 85.6 | 75.7 |
| November | 28 | 12.9 | 23.4 | 18.6 | 93.3 | 12.0 |
| December | 24.7 | 9.7 | 19.5 | 14.8 | 99 | 0.0 |

**Appendix 8.3 The monthly averages of climatic parameters at Gunganagar (S1), Saradanagar (S2) Gaindakot (S3) and Nahala (S4), Nepal, in 2005. (Source: HMRP, Rampur and Meteorological Station Pokhara).**

| Recording month | Study site | Atmospheric temperature ($^{o}$C) | | Soil temperature ($^{o}$C) | | Relative humidity (%) | Rainfall (mm) |
|---|---|---|---|---|---|---|---|
| | | Maximum | Minimum | Maximum | Minimum | | |
| January | S1 | 23.2 | 9.1 | 17.5 | 12.8 | 98.7 | 38.1 |
| | S2 | 23.2 | 9.1 | 17.5 | 12.8 | 98.7 | 38.1 |
| | S3 | 20.3 | 8.9 | 16.2 | 11.3 | 98.3 | 62.7 |
| | S4 | 18.2 | 7.8 | 15.3 | 10.0 | 89.2 | 19.5 |
| February | S1 | 26.7 | 10.3 | 20.5 | 14.4 | 97.9 | 0.0. |
| | S2 | 26.7 | 10.3 | 20.5 | 14.4 | 97.9 | 0.0 |
| | S3 | 26.7 | 10.3 | 19.8 | 13.7 | 97.9 | 0.0 |
| | S4 | 22.4 | 9.6 | 17.3 | 12.5 | 92.3 | 21.4 |
| March | S1 | 31.4 | 14.9 | 25.2 | 19.6 | 95.0 | 38.9 |
| | S2 | 31.4 | 14.9 | 25.2 | 19.6 | 95.0 | 38.9 |
| | S3 | 31.4 | 14.9 | 24.6 | 18.4 | 95.0 | 43.1 |
| | S4 | 25.4 | 13.9 | 22.3 | 13.0 | 93.4 | 65.5 |
| April | S1 | 35.9 | 21.6 | 29.3 | 22.1 | 68.1 | 133.5 |
| | S2 | 35.9 | 21.6 | 29.3 | 22.1 | 68.1 | 133.5 |
| | S3 | 36.2 | 22.4 | 28.7 | 20.4 | 65.1 | 21.4 |
| | S4 | 30.3 | 14.4 | 25.3 | 19.4 | 94.2 | 85.3 |
| May | S1 | 36.2 | 23.4 | 30.4 | 24.0 | 65.5 | 28.8 |
| | S2 | 36.2 | 23.4 | 30.4 | 24.0 | 65.5 | 42.5 |
| | S3 | 36.2 | 23.4 | 28.8 | 22.0 | 95.2 | 25.0 |
| | S4 | 32.5 | 18.5 | 27.5 | 21.3 | 94.2 | 102.0 |
| June | S1 | 36.6 | 25.1 | 31.0 | 24.8 | 71.7 | 133.9 |
| | S2 | 36.6 | 25.1 | 31.6 | 25.6 | 71.7 | 133.5 |
| | S3 | 36.6 | 25.1 | 30.0 | 26.6 | 96 | 133.5 |
| | S4 | 33.4 | 19.0 | 29.8 | 23.5 | 96 | 152.0 |
| July | S1 | 35.6 | 24.6 | 30.6 | 27.6 | 98 | 165.3 |
| | S2 | 35.6 | 24.6 | 30.6 | 27.6 | 98 | 165.3 |
| | S3 | 34.4 | 23.1 | 30.2 | 25.8 | 98 | 155.3 |
| | S4 | 34.5 | 21.5 | 29.6 | 25.3 | 98 | 185.0 |

# CHAPTER 9

## Monitoring studies of Scarabid beetles in different farming sites of Nepal

# Chapter 9

# Monitoring studies of Scarabid beetles in different farming sites of Nepal

## 9.1 SUMMARY

Because of the underground feeding habits, white grubs are more difficult to detect and control than many of the pests that feed on the above ground portion of the plants. If they are detected, identified, and abundances are understood, controlled measures may be applied at the appropriate time. Monitoring studies of adult beetles was carried out at different agro-ecological zones of Nepal to generate the basic information on pest dynamics. At the same time, the abundance of beetle species was studied through trapping different types of species. They were later identified to know the common species involved in the particular sites. A locally made light trap fitted with 125 w incandescent electric bulbs was operated for the trapping purpose. The studies have clearly indicated that, more than ninety different species of adult beetles are involved in cropping fields and considerable numbers of species occur as a overlapping generations. Normally, the chafer beetles in almost all the studied area were active during May to July coinciding with (near) crop sowing and harvesting stage in maize and (early) vegetative growth. In low belt (terai region) of Nepal, the flight of large number of beetles species occur in two peaks probably because of the short and overlapping generations of the larvae compared to mid hills regions. In mid hill areas, few species of beetles occur regularly in the same crop field, whereas, few other beetle species are common at alternate year probably because of the longer life cycle of the larvae. In 2003/04 in Gunganagar research site, the highest number of beetles were found in the month of May (5094) followed by April (2324), whereas, very few beetles were caught in Gaindakot research site. In this site, the highest number was 216 and 118 in the month of May and April respectively. Similar trend in the occurrence of the beetle was observed in 2005, however, with very low number of catches in Gaindakot research site. It is very interesting to note that more than forty different beetle species were recorded only within a single month of May in Gunganagar research site in 2005. Light traps are found one of the effective tools for monitoring phototropic beetles and the information generated from these studies are useful in planning the beetle management programme.

## 9.2 INTRODUCTION

The adult beetles of white grubs, Scarabaeid beetles, are distributed throughout the cropping site of Nepal. The larvae of these beetles are associated with majority of the crops and some time cause economic losses while in other cases they pose potential threat. Mainly the larvae of such beetles are associated in the major cereals, cash crops and other crops grown in upland area. In very few occasions, the adult beetles are reported to be as crop pests (farmer's discussion, 2004), however, many species of white grubs are becoming problematic across the country. At the same time their dynamics in terms of seasonal abundances, species involvement and precise identification is severely lacking in the country. Until recent past, only very few insect species such as *Phyllophaga rugosa*, *Holotrichia* spp and *Anomala* spp (Joshi, 1994; Neupane, 1993) were reported as major species in Nepal; however, recent studies have indicated several species are involved within the same locality and majority of them involve in crop damages. Adult *Popillia* spp have

also been reported to attack finger millet panicles (Pandey *et al.* 1995) but the role of soil borne larvae have not been considered yet. This situation is mainly due to lack of initiation of systematic works throughout the country.

The adults of white grubs can be monitored using light traps as well as sampling through the soil. Light traps are useful tools for monitoring the phototropic insects including adult beetles of white grubs. Monitoring primarily gives the basis for deciding and formulating management strategy against pest insects. Various methods of insect pest monitoring are designed elsewhere; however, almost negligible works are done so far in Nepal. Curative controls are primarily dependent using synthetic chemicals without understanding the bio-ecology of the pest insects. Pandey *et al.* (1993) reported *Phyllophaga* spp are the major component of white grubs in Nepal based on the monitoring works conducted at Lumle Centre (1675 m asl; 28° 18' N and 83° 49' E). He further reported that their high activity occurs during June-July however, there is lack of information about the seasonal occurrence of the different species of white grubs. Furthermore, identification of the damaging species of white grubs is severely lacking throughout the country. Insect pest monitoring is therefore, one of the important areas of entomological study to understand the pest dynamics in relation to strategic management. The findings of the studies conducted during different seasons in different parts of farmer's field are presented hereunder.

## 9.3 MATERIAL AND METHODS

Monitoring studies of adult beetles through light trap was undertaken in two consequent seasons and in different places between the seasons. Initially, in 2003/04, it was carried out in five different agro-ecological zones however, in 2004/05, it was limited to only two locations. The monitoring studies include the farming site as well as river basin forest site with different altitudes. The study sites were varied in terms of difference in vegetation as well as soil characteristics. It was hoped that maximum variation of the adult beetle species would be obtained during monitoring.

Figure 9.1 Light trap operation in Gunganagar (left) and Gaindakot (right) Nepal.

During monitoring, a light trap with a 125 watt incandescent electric bulb was operated in farmers' field. The light traps were operated once a week from the dusk of previous day to the dawn of next day. In some sites, power failure was occurred in the day of light trap operation; therefore, light trap in such situation was run immediately of the next day as a compensation day. Insects attracted to the light traps were collected into a poly pot through a funnel trap fitted just beneath the electric light. A cotton swab with ethyl acetate was used as a killing agent inside the light trap. In the next morning, the trappings were grouped firstly among beetle and non-beetles, however, counting and preservation was done only to the beetle species.

### 9.3.1 Monitoring of beetles during 2003 and 2004

Monitoring was carried out in five different agro-ecological zones such as, Gunganagar and IAAS Rampur (Chitwan District), Gaindakot (Nawalparasi District), Rishing Patan (Tanahun, District) and Pang (Parbat, District) of Nepal. Monitoring studies in these sites was carried out from November 2003 through December 2004. The collected beetles were preserved into 95% ethanol with their locality tag. Later on, they were brought into the Department of Entomology, Insect Pathology Unit of IAAS, Rmapur and sub-grouped into dung beetles (Coprinae) and other beetles. The dung beetles were not counted and preserved in the reference collection; however, other beetles were grouped into Cetoninae, Dynastinae, Melolonthinae, Rutelinae and other sub-families.

Table 9.1 Characteristics of the study sites of different parts of Nepal with monitoring of adult beetles through light trap during November 2003 through October 2005.

| Site | District/Zone/ Region | Domains | Geographical coordination | Cropping pattern | Soil type |
|---|---|---|---|---|---|
| Gunganagar | Chitwan Dist, Narayani Zone, Central region | Low hill, terai | 27°39'N, 84°19E<br>230 m asl | Cropping site<br>(Maize-maize-vegetables) | Sandy soil, moderate fertile |
| Rampur | Chitwan Dist, Narayani Zone, Central region | Low hill, terai | 27°39'N, 84°21E<br>230 m asl | Cropping site<br>(Maize-maize-vegetables) | Sandy, moderately fertile |
| Gaindakot | Nawalparasi Dist, Narayani Zone, Central region | Low hill, terai | 27°42.5'N, 84°25E<br>150 m asl | Forest river basin<br>(Maize-maize-vegetables) | Sandy loam, fertile |
| Nahala | Tanahun Dist, Gandaki Zone, Western, region | Mid hill, tar area | 28°11.5'N, 83°21E<br>950 m asl | Cropping site (Maize-upland rice-vegetables) | Red loam soil, fertile |
| Rishing Patan | Tanahun Dist, Gandaki Zone, Western region | Mid hill, tar area | 27°46'N, 85°37E<br>350 m asl | Cropping site, river basin (Maize-legumes-tomato) | Sandy loam, fertile |
| Pang | Parbat Dist, Dhaulagiri Zone, Western region | Mid high hill, tar area | 28°15'N, 83°37E<br>1155 m asl | Cropping site<br>(Maize-millet-fallow) | Red clay, fertile |

### 9.3.2 Monitoring of beetles during 2005

Unlike previous season of 2003/04, monitoring study in 2005 was limited only into two sites, namely, Gaindakot and Gunganagar of Nawalparasi and Chitwan District respectively. Because of the security situation of the country the former three sites such as Pang, Rishing Patan and Nahala were abandoned. However, similar method was adopted for the collection, preservation and identification of the beetle species for entire season of study. In these sites, monitoring was started from the month of November 2003 through the end of October 2005. The beetles were later identified by Prof. Dr. Peter Nagel and Dr. Dirk Arhens based on the morphology and identification keys of the insects. The identification keys were followed as per their instruction for the whole process of identification. The identified insects are maintained as reference collection at IAAS, Rampur, Chitwan, Nepal.

### 9.3.3 Phenology of beetles during 2003-2005

In order to understand the general phenology of the adult beetles, all the trapped specimens were counted on weekly basis. This information was later converted into the understanding of the seasonal abundance of the beetle specimens and species per month over the period of 2003 through 2005. The same methodology was followed for the trapping, preservation, counting and identification of the specimens.

## 9.4 RESULTS

### 9.4.1 Monitoring of beetles during 2003 and 2004

The findings of the monitoring study conducted during 2003/04 is summarised in Tables 9.2 and 9.3. The species composition (Table 9.2) has clearly indicated that Melolonthinae are the highest followed by Rutellinae. The Cetoninae are the least distributed groups across the study sites. Among these taxa should be also taxa with “Pest” status. The composition of adult beetle species which fall under four sub-families is presented in Table 9.2.

Table 9.2 Species composition, species number and total specimens collected through light traps since November 2003 - October 2004 in different sites of Nepal (the number presented in the table are collected over 48 trap nights where light trap was operated 1 night per week).

| **Species composition of the beetles** | **Adult beetle species collected in different sites of Nepal in 2003/04** | | | | | **Sub family** |
|---|---|---|---|---|---|---|
| | Gaindakot | Gunganagar | IAAS Rampur | Pang | Rising Patan | |
| *Chiloloba acuta* Wiedemann | - | 2 | - | 7 | - | Cetoniinae |
| *Dicranocephalus wallichi wallichi* Hope | 1 | - | - | - | - | Cetoniinae |
| *Oxycetonia histro* (Oliver) | - | 3 | - | - | - | Cetoniinae |
| *Oxycetonia variicolor* (F.) | - | 1 | - | - | - | Cetoniinae |
| *Alissonotum binodulum* Fairmaire | 5 | 45 | - | - | - | Dynastinae |
| *Alissonotum simile* Arrow | 37 | 39 | 5 | - | - | Dynastinae |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Eophileurus forsteri* Endroedi | - | - | - | - | 1 | Dynastinae |
| *Heteronychus liioderes* Redtenbacher | 8 | 79 | - | - | - | Dynastinae |
| *Heteronychus* sp. 2 | 7 | 3 | - | - | - | Dynastinae |
| *Peltonotus morio* Burmeister | - | 1 | 1 | - | - | Dynastinae |
| *Pentodon algerinum indicum* Endroedi | - | 2 | - | - | - | Dynastinae |
| *Phyllognathus dionysius* F. | - | - | - | 10 | 10 | Dynastinae |
| *Xylotrupes gideon* L. | 2 | - | - | - | 1 | Dynastinae |
| *Apogonia* sp. 1 | - | - | - | 1 | - | Melolonthinae |
| *Asactopholis dehradunensis* Mittal | - | - | - | 6 | - | Melolonthinae |
| *Cyphochilus pygidialis* Nonfried | - | - | - | - | - | Melolonthinae |
| *Hemiserica nasutella* Ahrens | - | 3 | - | - | - | Melolonthinae |
| *Holotrichia anthracina* Brenske | - | - | - | - | 1 | Melolonthinae |
| *Holotrichia nigricollis* Brenske | - | - | - | 311 | 9 | Melolonthinae |
| *Holotrichia pruinosa* Wied. | - | - | - | 1 | 2 | Melolonthinae |
| *Holotrichia seticollis* Moser | 1 | 6 | 1 | 5 | 6 | Melolonthinae |
| *Holotrichia sikkimensis* Brenske | - | - | - | 35 | 4 | Melolonthinae |
| *Holotrichia* sp. 2 | - | - | - | 153 | 25 | Melolonthinae |
| *Idionychus excisa* Arrow | - | 61 | 41 | - | - | Melolonthinae |
| *Lepidiota albistigma* Burmeister | - | - | - | 1 | - | Melolonthinae |
| *Maladera affinis* (Blanchard) | 41 | 105 | 13 | - | - | Melolonthinae |
| *Maladera cardoni* (Brenske) | 3 | 69 | - | - | - | Melolonthinae |
| *Maladera iridescens* (Blanchard) | - | - | - | 1 | - | Melolonthinae |
| *Maladera pokharae* Ahrens | - | - | - | 1 | - | Melolonthinae |
| *Maladera quinquidens* (Brenske) | - | - | - | 14 | 1 | Melolonthinae |
| *Maladera schenklingi* (Moser) | 1 | - | - | - | - | Melolonthinae |
| *Maladera thomsoni* (Brenske) | - | - | 1 | 725 | 26 | Melolonthinae |
| *Melolontha (Hoplosternus?) indica* Hope | - | - | - | - | - | Melolonthinae |
| *Schizonychia fuscescens* Blanchard | 2 | 12 | 1 | - | 2 | Melolonthinae |
| *Sophrops cardoni* Brenske | - | - | - | 1 | - | Melolonthinae |
| *Sophrops* spec. 1 | 4 | 8 | 46 | 18 | - | Melolonthinae |
| *Sophrops* spec. 2 | - | - | - | - | 4 | Melolonthinae |
| *Sophrops* spec. 3 | - | - | - | 130 | - | Melolonthinae |
| *Sophrops* spec. 5 | - | 2 | 1 | 3 | - | Melolonthinae |
| *Tetraserica ferrugata* (Blanchard) | - | - | - | 1 | - | Melolonthinae |
| *Adoretosoma bruschii* Sabatinelli | - | - | - | - | 1 | Rutelinae |
| *Adoretus coronatus* Burmeister | - | 1 | - | - | - | Rutelinae |
| *Adoretus lasiopygus* Burmeister | 9 | 1087 | 2 | 6 | - | Rutelinae |
| *Adoretus serripes* Arrow | - | 16 | 1 | - | - | Rutelinae |
| *Adoretus simplex* Sharp | 1 | 7 | - | - | - | Rutelinae |
| *Adoretus* sp. 10 | - | 1 | - | - | - | Rutelinae |
| *Adoretus* sp. 11 | - | 1 | - | - | - | Rutelinae |
| *Adoretus* sp. 2 | - | 21 | - | 1 | - | Rutelinae |
| *Adoretus* sp. 9 | - | 1 | - | - | - | Rutelinae |
| *Adoretus versutus* Harold | 3 | 27 | 1 | - | - | Rutelinae |
| *Adorrhinyptia dorsalis* Burm. | - | 2 | - | - | - | Rutelinae |
| *Anomala bengalensis* Blanchard | 1 | 6 | - | - | 1 | Rutelinae |
| *Anomala bilobata* Arrow | 7 | 53 | 2 | 246 | 52 | Rutelinae |
| *Anomala cantori* (Hope) | 16 | 62 | 7 | 4 | - | Rutelinae |
| *Anomala* cf. *biharensis* Arrow | - | 1 | - | - | - | Rutelinae |
| *Anomala chlorosoma* Arrow | - | 1 | - | - | - | Rutelinae |
| *Anomala comma* Arrow | 1 | 1 | - | 11 | - | Rutelinae |
| *Anomala dimidiata* (Hope) | 25 | 133 | 42 | 32 | 1 | Rutelinae |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Anomala euops* Arrow | - | 2 | - | - | - | Rutelinae |
| *Anomala marginipennis* Arrow | - | - | - | 78 | 2 | Rutelinae |
| *Anomala perplexa* Hope | 2 | - | - | - | - | Rutelinae |
| *Anomala* sp. n. 1 | - | 1 | - | - | - | Rutelinae |
| *Anomala* sp. n. 2 | 1 | 106 | 4 | - | - | Rutelinae |
| *Anomala* sp. n. 3 | 1 | - | - | - | - | Rutelinae |
| *Anomala testacea* Hope | - | - | - | 1 | 1 | Rutelinae |
| *Anomala varicolor* (Gyllenhal) | 1 | 11 | 2 | 89 | 13 | Rutelinae |
| *Anomala variegata* Hope | 8 | 13 | 7 | 25 | 4 | Rutelinae |
| *Anomala xanthoptera* Blanchard | 3 | 105 | 120 | - | - | Rutelinae |
| *Mimela bicolor* Hope | - | - | - | 1 | - | Rutelinae |
| *Mimela* cf. *fulgidivittata* Blanchard | - | 2 | - | 2 | - | Rutelinae |
| *Mimela horsfieldii* Hope | - | - | - | - | - | Rutelinae |
| *Mimela inscripta* (Nonfried) | - | 1 | - | - | - | Rutelinae |
| *Mimela sericea* Ohaus | - | - | - | - | - | Rutelinae |
| *Mimela siliguria* Arrow | 5 | 9 | - | - | - | Rutelinae |
| *Mimela* sp. n. (close to *M. decipiens*) | - | 6 | - | - | - | Rutelinae |
| *Parastasia rufopicta* Westwood | - | 1 | - | - | - | Rutelinae |
| *Popillia birmanica* Arrow | - | 4 | - | - | - | Rutelinae |
| *Rhamphadoretus suillus* Arrow | - | 4 | - | - | - | Rutelinae |
| **Species number** | **27** | **47** | **19** | **31** | **21** | |
| **Specimens number** | **196** | **2127** | **298** | **1920** | **167** | |

The result has showed that the highest numbers of species were involved in Gunganagar (47) research sites followed by Pang (31) and other sites. In Chitwan, large numbers of beetles were found within the group of *Maladera*, *Adoretus*, *Heteronychus*, *Anomala* etc. Similarly, *Holotrichia*, *Sophrops*, *Anomala* and other kind of *Maladera* are common in the high mid hills of Parbat farming sites. Whereas, in high hills *Lepidiota*, *Mimela* and *Anomala* are among the common species. Very interestingly *Anomala* groups are cosmopolite species found across the study sites.

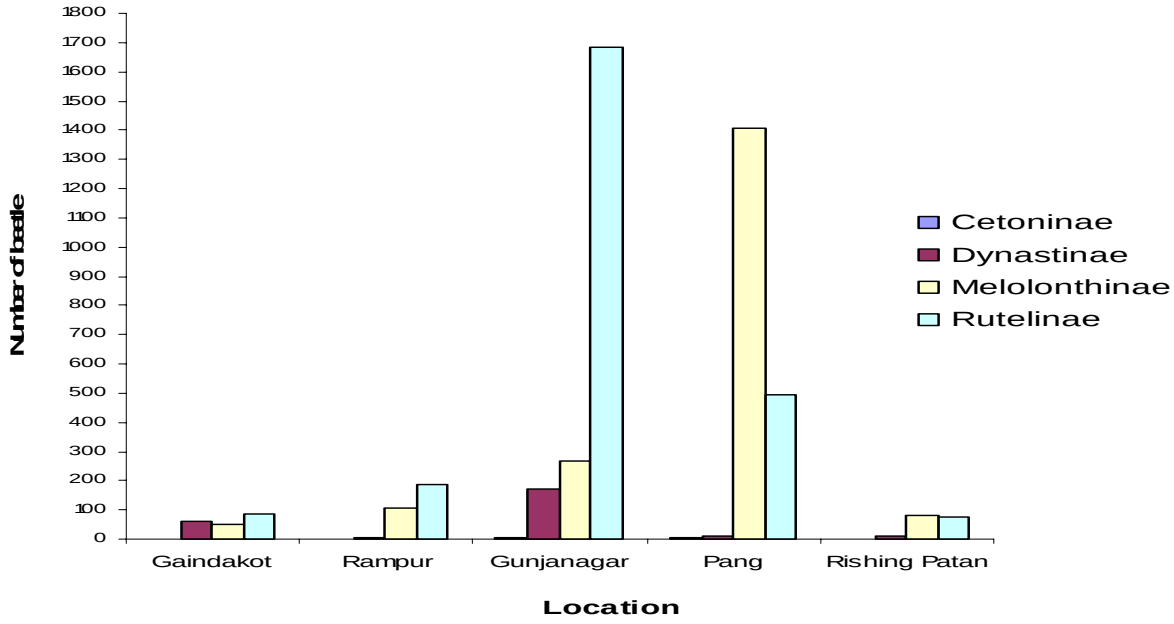



Figure 9.2 Occurrence of different beetle subfamilies in different locations in 2003-2004.

In order to know the most abundant species in the study sites their percentage was determined from the same study. The most common species of the beetles based on sub-family level are Scarabaeinae, Melolonthinae, Rutelinae, Dynastinae and Cetoninae (Figure 9.2).

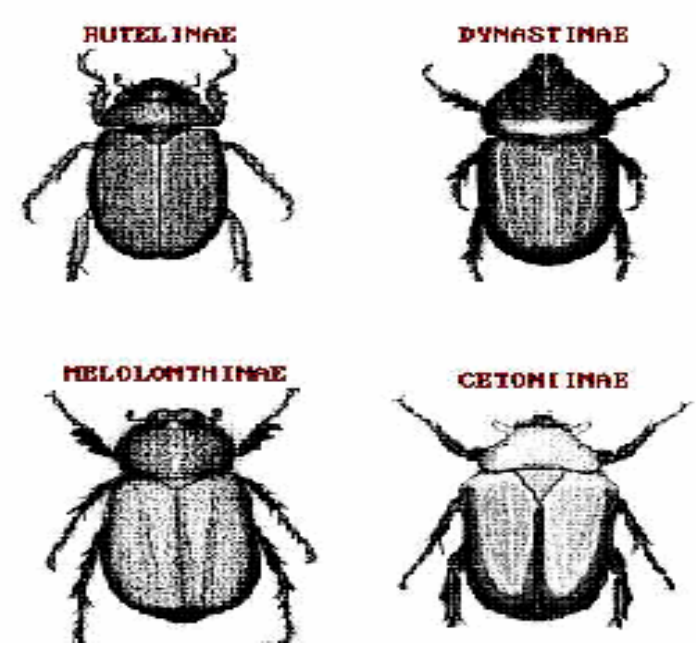



Figure 9.3 Most common adult beetles at sub-family level in the study sites.

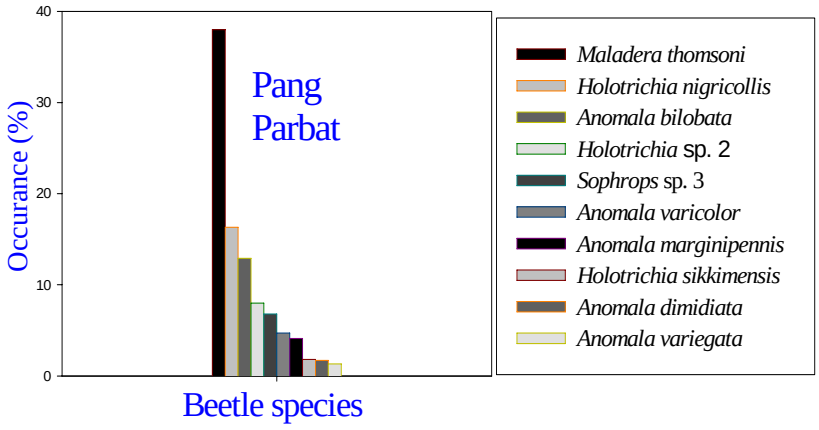
However, other species occurred more or less in frequent and the top most ten common species recorded over the period of 2003/04 are presented in table 9.3.

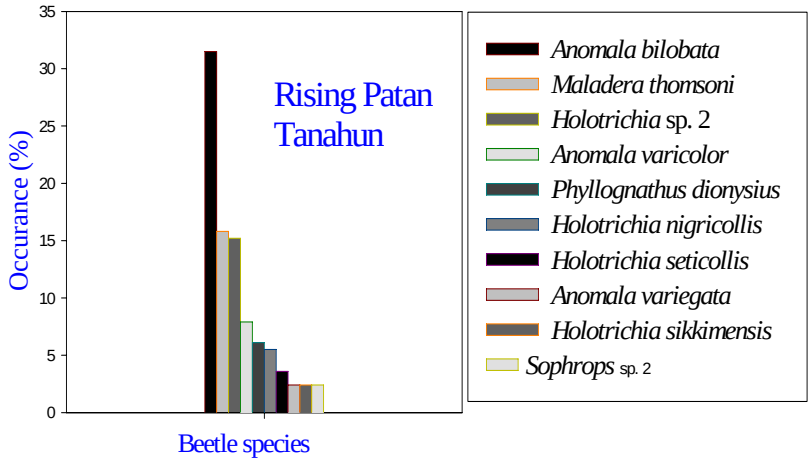
Table 9.3 Top ten most abundant species during light trap study since November 2003-December 2004 in different sites of Nepal (the occurrence percentage presented in the table are the collections of 48 trap nights where light trap was operated 1 night per week). Among these taxa should be also taxa with "pest" status, where X indicates the pest status based on the references.

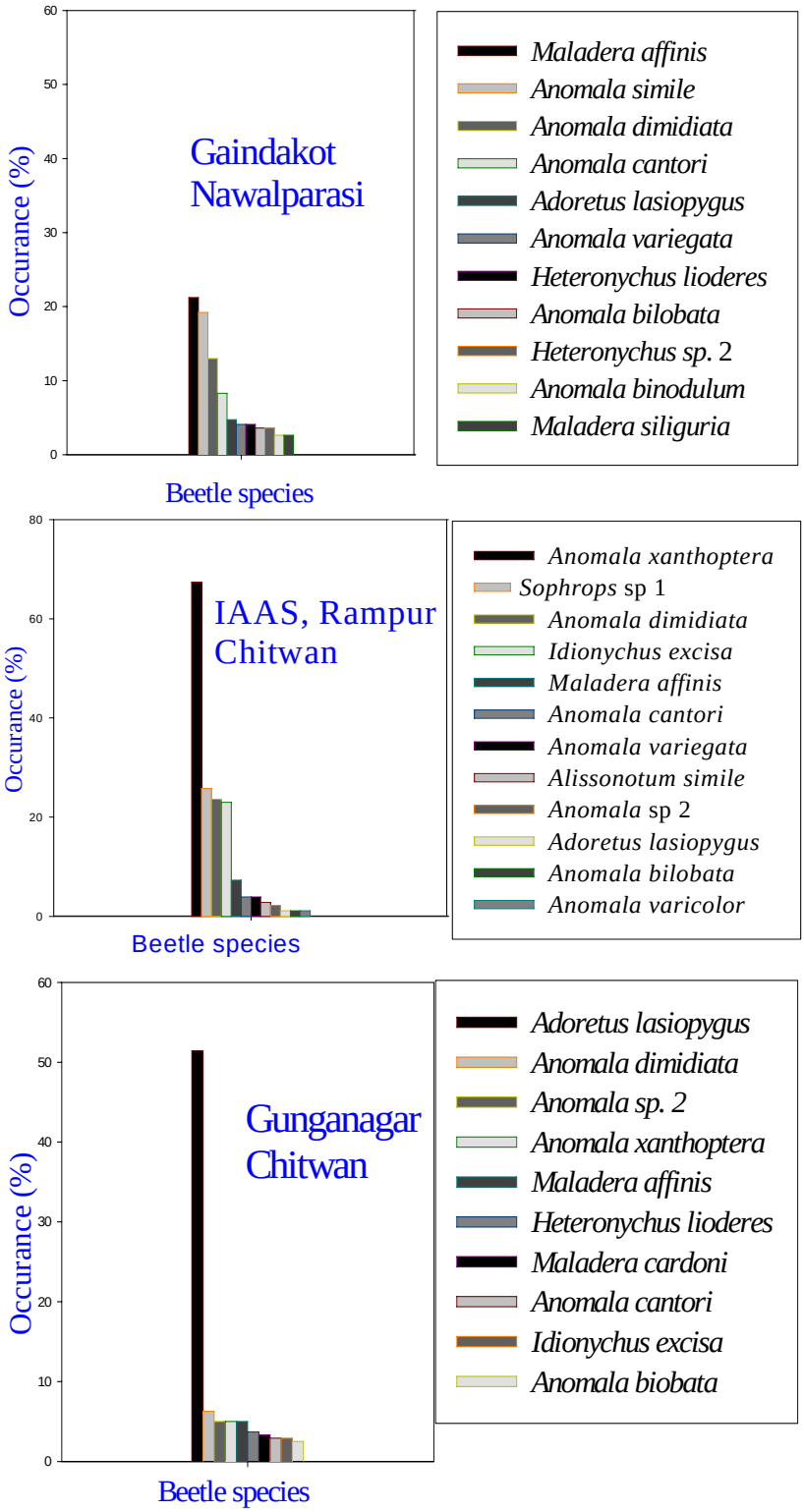
| **Gaindakot, Nawalparasi** | **Occurrence percentage** | **Pests status** |
|---|---|---|
| *Maladera affinis* Blanchard | 21.2 | X |
| *Alissonotum simile* Arrow | 19.2 | X |
| *Anomala dimidiata* (Hope) | 13.0 | |
| *Anomala cantori* (Hope) | 8.3 | |
| *Adoretus lasiopygus* Burmeister | 4.7 | X |
| *Anomala variegata* Hope | 4.1 | |
| *Heteronychus lioderes* Redtenbacher | 4.1 | X |
| *Anomala bilobata* Arrow | 3.6 | |
| *Heteronychus* sp. 2 | 3.6 | |
| *Alissonotum binodulum* Fairmaire | 2.6 | |
| *Mimela siliguria* Arrow | 2.6 | |

| | | |
|---|---|---|
| **Gunganagar, Chitwan** | | |
| *Adoretus lasiopygus* Burmeister | 51.4 | X |
| *Anomala dimidiata* (Hope) | 6.3 | |
| *Anomala* sp. n. 2 | 5.0 | |
| *Anomala xanthoptera* Blanchard | 5.0 | |
| *Maladera affinis* (Blanchard) | 5.0 | X |
| *Heteronychus lioderes* Redtenbacher | 3.7 | X |
| *Maladera cardoni* (Brenske) | 3.3 | |
| *Anomala cantori* (Hope) | 2.9 | |
| *Idionychus excisa* Arrow | 2.9 | |
| *Anomala bilobata* Arrow | 2.5 | |
| **IAAS-Rampur, Chitwan** | | |
| *Anomala xanthoptera* Blanchard | 67.4 | |
| *Sophrops* spec. 1 | 25.8 | |
| *Anomala dimidiata* (Hope) | 23.6 | |
| *Idionychus excisa* Arrow | 23.0 | |
| *Maladera affinis* (Blanchard) | 7.3 | X |
| *Anomala cantori* (Hope) | 3.9 | |
| *Anomala variegata* Hope | 3.9 | |
| *Alissonotum simile* Arrow | 2.8 | X |
| *Anomala* sp. n. 2 | 2.2 | |
| *Adoretus lasiopygus* Burmeister | 1.1 | X |
| *Anomala bilobata* Arrow | 1.1 | |
| *Anomala varicolor* (Gyllenhal) | 1.1 | |
| **Pang, Parbat** | | |
| *Maladera thomsoni* (Brenske) | 38.0 | X |
| *Holotrichia nigricollis* Brenske | 16.3 | |
| *Anomala bilobata* Arrow | 12.9 | |
| *Holotrichia* sp. 2 | 8.0 | |
| *Sophrops* spec. 3 | 6.8 | |
| *Anomala varicolor* (Gyllenhal) | 4.7 | |
| *Anomala marginipennis* Arrow | 4.1 | |
| *Holotrichia sikkimensis* Brenske | 1.8 | |
| *Anomala dimidiata* (Hope) | 1.7 | |
| *Anomala variegata* Hope | 1.3 | |
| **Rishing Patan, Tanahun** | | |
| *Anomala bilobata* Arrow | 31.5 | |
| *Maladera thomsoni* (Brenske) | 15.8 | X |
| *Holotrichia* spec. 2 | 15.2 | |
| *Anomala varicolor* (Gyllenhal) | 7.9 | |
| *Phyllognathus dionysius* F. | 6.1 | X |
| *Holotrichia nigricollis* Brenske | 5.5 | |
| *Holotrichia seticollis* Moser | 3.6 | |
| *Anomala variegata* Hope | 2.4 | |
| *Holotrichia sikkimensis* Brenske | 2.4 | |
| *Sophrops* spec. 2 | 2.4 | |

Table 9.3 has clearly revealed the variation of the dominance of the species from one agro-environment to another agro-environment. In Gaindakot research site, major dominance of the beetle species was *M. affinis* (21.2%) followed by *A. simile* (19.2%) and *M. siliguria* were the lowest (2.6%) in occurrence. In another farming site, Gunganagar, Chitwan, *Ad. lasiopygus* was found one of the dominant species (51.4%) and interestingly rest of other species were not above than 6%. The third light trap study inside the Rampur Campus revealed the highest (67.4%) occurrences of *An. Xanthoptera* followed by *Sophrops* sp. 1 (25.8%) and rest were below than 10%. In contrasts to these low hill (terai) sites, the beetle species in mid hill research site, Rishing Patan, Tanahun showed the highest (31%) occurrence of *An. bilobata* followed by *M. thomsoni* and *Holotrichia* sp. 2 (15.8%) whereas, rest of the species were less than 5%. Interestingly, the major species of the beetles were different in Parbat (mid high hill) research site than the rest of the sites. In this site, *M. thomsoni* was by far the highest (38%) number than the rest of the species. The second most abundant species was *H. nigricolis* (16.3%) and rest of the beetle species were within the range of 10%. In total more than 4700 specimens were examined representing so far recognized 78 species. The occurrence of the most abundant beetle species recorded within the period of 2003/04 in five different research field is presented in below mentioned Figures 9.4-9.8.






Figures 9.4-9.8 Most abundant beetle species as collected by light traps since November 2003 –December 2004 in five agro-ecological zones Nepal (the occurrence percentage presented in the table are the collections of 48 trap nights where light trap was operated 1 night per week).

### 9.4.2 Monitoring of beetles during 2005

Unlike monitoring studies of 2003/04, the monitoring in 2005 showed different trend of the occurrence of beetle species. In Gunganagar research site, *Anomala* sp. n. 2 was found one of the dominant species (453) followed by *An. xanthoptera* (214) and *Ad varicolor* (186), whereas, in previous season, *Ad. lasiopygus* was one of the dominant (51.4%) species. During this year, some of the new species were also recorded. For example *Idionychus excise* and *An. beharensis* and *Mimela inscripta* were some of them. The repeated occurrence of similar species and some of the new species clearly suggests the dominance of some beetle species leaving others as minor species. It is interesting to note that, some species of beetle such as *H. seticollis* and *Al. simile* occurred during winter months, however, the occurrences of majority of other beetle species confined during April through July. Second week of the May was found as the peak period of beetle occurrence where only *An. xanthoptera* were trapped (347 per night) (Table 9.4). This study therefore, has convincingly revealed that, most of the egg laying coincides during the preparation of maize fields in Nepal where early stage seedlings are highly prone to be attacked by grown up beetle larvae. In addition to this, this study has also indicated the possibility of the involvement of many more species within the short geographysical distance and regular occurrences of the larvae in the same field. This situation has necessitated the regular control practices.

Similar trend of the occurrences of beetle species was found in Gaindakot with that of Gunganagar research site. Very interestingly, two of the beetle species such as *H. seticollis* and *Al. simile* occurred in the winter month following the same pattern of Gunganagar site. In this site, *An. xanthoptera* was dominant (176) followed by *An. dimidiate* (55). However, in previous season i. e. 2003/04 collections, *M. affinis* and *Al. simile* were by far the highest (21.2% and 19.2% respectively) proportion. There is one very clear similarity across the study sites i.e. occurrence of the most of the beetle species was found during the period of April through July following the similar pattern of previous year. Furthermore, some of the new beetle species such as *Apogoina* sp. 1, *Adorrhinyptia dorsalis*, *Oxycetonia* sp. and *X. gideon* were also found involved in this site, which were not found in previous season of collection. This evidence suggests the involvement of numerous species of the beetles in both of the research sites (Table 9.5).

The distribution of the beetle species in the study sites is presented in the Figures 9.9 and 9.10 for Gunganagar and Gaindakot research sites respectively. The total numbers of species involvement in both the sites were thirty two however occurrences in the number of specimens was considerably higher in the former sites as compared to later site.

Table 9.4 Species composition, species number and total specimens collected through light traps since January - November 2005 in Gunganagar, Chitwan, Nepal (the number presented in the table are collected over 41 trap nights where light trap was operated 1 night per week).

| | Month of collection (weekly basis) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | January | | | | February | | | | March | | | | April | | | | May | | | | June | | | | July | | | | August | | | | September | | | | October | | | | November | Total |
| Beetle species | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 night | |
| *Anomala* sp. n. 2 | | | | | | | | | | | | | | | | 3 | | 9 | 347 | 92 | 1 | | | | | | | | 1 | | | | | | | | | | | | | 453 |
| *Anomala xanthoptera* | | | | | | | | 1 | 20 | 32 | 42 | 62 | 54 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | | 214 |
| *Anomala varicolor* | | | | | | | | | | | | | | | | 14 | 41 | 99 | 32 | | | | | | | | | | | | | | | | | | | | | | | | 186 |
| *Adoretus lasiopygus* | | | | | | | | | | | | | | | | | | 42 | | 6 | 1 | 1 | 4 | 25 | 9 | 14 | 19 | 23 | 4 | 14 | 9 | 3 | 1 | 2 | | | 2 | 1 | | | | | 180 |
| *Maladera affinis* | | | | | | | | | | | | 6 | | | 3 | 6 | 28 | 14 | 19 | | 12 | 1 | 7 | 1 | 2 | 3 | 1 | 1 | 1 | | 6 | 10 | 1 | 6 | 3 | 4 | 2 | | 1 | | 5 | | 143 |
| *Heteronychus* sp. 2 | | | | | | | | | | | | | | | | | 2 | 7 | | 12 | | | 61 | | | | | | | | | | | | | | | | | | | | 82 |
| *Maladera cardoni* | | | | | | | | | | | | | | | | | | 8 | 23 | | 43 | | 3 | | | | | | | 1 | | 1 | | | | | | | | | | 79 |
| *Anomala dimidiata* | | | | | | | | | | | | | | | | | | | 2 | 1 | 1 | | 18 | 10 | 1 | 7 | | 4 | 17 | 3 | | 3 | | 1 | 1 | | | | | 1 | | 70 |
| *Schizonychia fuscescens* | | | | | | | | | | | | | | | | | 1 | 6 | 53 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 62 |
| *Idionychus excisa* | | | | | | | | | | | 1 | 3 | 10 | 4 | 4 | 8 | 8 | | 4 | | | | 1 | | | | | | | | | 1 | | | 1 | | | 1 | | | | | 46 |
| *Anomala variegata* | | | | | | | | | | | | | 2 | 12 | 1 | 1 | | | | | | | 1 | | | | 1 | 2 | | 1 | | | | | | 1 | 2 | 1 | 4 | 3 | | 32 |
| *Holotrichia seticollis* | 2 | | | 1 | | | | | | | | | | | | 2 | 3 | | 9 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 19 |
| *Adoretus versutus* | | | | | | | | | | | | | | | 3 | | 6 | | | | | | | 1 | 1 | | 1 | | | | | 2 | | | 2 | | | | | | | 16 |
| *Alissonotum simile* | | | | | | | 3 | | 2 | 3 | | | | | 1 | | | | | | | | 6 | | | | | | | | | | | | | | | | | | | 15 |
| *Adoretus* sp. 2 | | | | | | | | | | | | | | | | | | | 7 | | 6 | | 1 | 1 | | | | | | | | | | | | | | | | | | | 15 |
| *Heteronychus lioderes* | | | | | | | | | | | | | ? | | | | 3 | | 1 | 1 | | | 9 | | | | | | | | | | | | | | | | | | | | 14 |
| *Anomala dorsalis* | | | | | | | | | | | | | | | | | | | | | 2 | 11 | | | | | | | | | | | | | | | | | | | | | 13 |
| *Alissonotum binodulum* | | | | | | | | | | | | | 3 | 3 | 1 | | | | | 2 | | | 1 | | | | | | | | | 1 | | | | 1 | | | | | | | 12 |
| *Anomala testacea* | | | | | | | | | | | | | 2 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | 6 |
| *Maladera thomsoni* | | | | | | | | | | | | | | | 3 | | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| *Sophrops* sp. 5 | | | | | | | | | | | | | | | | | | | | | | | | 1 | | 1 | 1 | 2 | | | | | | | | | | | | | | | 5 |
| *Anomala bilobata* | | | | | | | | | | | | | | | | | | 5 | | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| *Maladera quinquidens* | | | | | | | | | | | | | | | | | | | 1 | | 2 | | 1 | | | | | | | | | | | | | | | | | | | | | 4 |
| *Anomala cantori* | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | 1 | | 1 | | | | | 1 | | | | | | | | 4 |
| *Anomala biharensis* | | | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| *Maladera brevistylis* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Adoretus serripes* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | 1 |
| *Adoretus* sp. 11 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | 1 |
| *Adoretus* sp. 13 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | 1 |
| *Adoretus* sp. 14 | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| *Anomala bengalensis* | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Mimela inscripta* | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | 1 |

Table 9.5 Species composition, species number and total specimens collected through light traps since January - November 2005 in Gaindakot, Nawalparasi, Nepal (the number presented in the table are collected over 41 trap nights where light trap was operated 1 night per week).

| | Month of collection (weekly basis) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | January | | | | February | | | | March | | | | April | | | | May | | | | June | | | | July | | | | August | | | | September | | | | October | | | | November | Total |
| Beetle species | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 night | |
| *Anomala xanthoptera* | | | 21 | | 5 | 9 | | 47 | 21 | 36 | 30 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | | 176 |
| *Anomala dimidiata* | | | | | | | | | | | | | | | | | | | | | 28 | 2 | | 1 | 1 | 2 | 11 | 9 | 1 | | | | | | | | | | | | | | 55 |
| *Anomala* sp. n. 2 | | | | | | | | | | | | | | 2 | 3 | | 22 | 6 | 20 | | | | | | | | | | | | | | | | | | | | | 1 | | | 54 |
| *Maladera affinis* | | | | | | | | | | | | 1 | | 13 | 14 | | 1 | 1 | | 1 | 2 | | | 1 | | 1 | 2 | | | | | 2 | | 2 | 2 | | | 1 | 2 | | | | 46 |
| *Adoretus lasiopygus* | | | | | | | | | | | | | | | | | | | | | 3 | 4 | | 2 | | 3 | 5 | 9 | | | | 4 | | 2 | 3 | 1 | | 1 | 2 | 1 | | 1 | 41 |
| *Anomala variegata* | | | | | | | | | | | | 17 | 2 | 2 | | 1 | | | | | 1 | | 1 | 2 | | | 1 | 2 | | | | | | | | 1 | 3 | 1 | 3 | | 1 | 38 |
| *Idionychus excisa* | | | | | | | | 2 | | | | 1 | 1 | 9 | 2 | 2 | | 2 | 1 | 2 | | | | | | | | | | | | | | | | | | | 1 | | | 23 |
| *Holotrichia seticollis* | 2 | | | | | | | | | | | | 1 | 2 | 2 | 3 | | 2 | | | 1 | | | | | | | | | | | | | | | | | | 1 | | | 14 |
| *Sophrops* sp. 5 | | | | | | | | | | | | | | | | | | | 2 | | | 2 | 3 | | 2 | 2 | | | | | 1 | | | | | | | | | | | 12 |
| *Anomala varicolor* | | | | | | | | | | | | 5 | | | | 1 | | 3 | 1 | | | | | | | | | | | | | | | | | | | | | | | 10 |
| *Adoretus versutus* | | | | | | | | | | | | | | 3 | | 4 | | | | | | | | | 1 | | | | | | | | | | | | | | | | | 8 |
| *Alissonotum simile* | | | | 2 | | | 3 | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 7 |
| *Maladera cardoni* | | | | | | | | | | | | | | | | 1 | 1 | | | 1 | | | | 1 | | 1 | | | | | | | | 1 | | | | | | | | 6 |
| *Schizonychia fuscescens* | | | | | | | | | | | | | | | | | 1 | 4 | | | | 1 | | | | | | | | | | | | | | | | | | | | 6 |
| *Mimela siliguria* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | | | 2 | | | | 6 |
| *Alissonotum binodulum* | | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | | | 1 | 1 | 1 | | | | | | | | | | | 5 |
| *Adoretus* sp. 11 | | | | | | | | | | | | | | | | | | 5 | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| *Anomala testacea* | | | | | | | | | | | | 2 | | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| *Anomala bilobata* | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | | 1 | | | | | | | | | | | | | | | 3 |
| *Anomala biharensis* | | | | | | | | | | | | | | | | | | 1 | 1 | | | 1 | | | | | | | | | | | | | | | | | | | | 3 |
| *Heteronychus lioderes* | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 2 |
| *Apogonia* sp. 1 | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | | | | 2 |
| *Maladera thomsoni* | | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| *Adorrhinyptia dorsalis* | | | | | | | | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | | | | | | 2 |
| *Anomala cantori* | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | 2 |
| *Anomala chlorosoma* | | | | | | | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | | | | | | | 2 |
| *Heterorrhina micans* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | 1 |
| *Oxycetonia* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| *Heteronychus* sp. 2 | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Xylotrupes gideon* | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | 1 |
| *Maladera quinquidens* | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Anomala bengalensis* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | 1 |

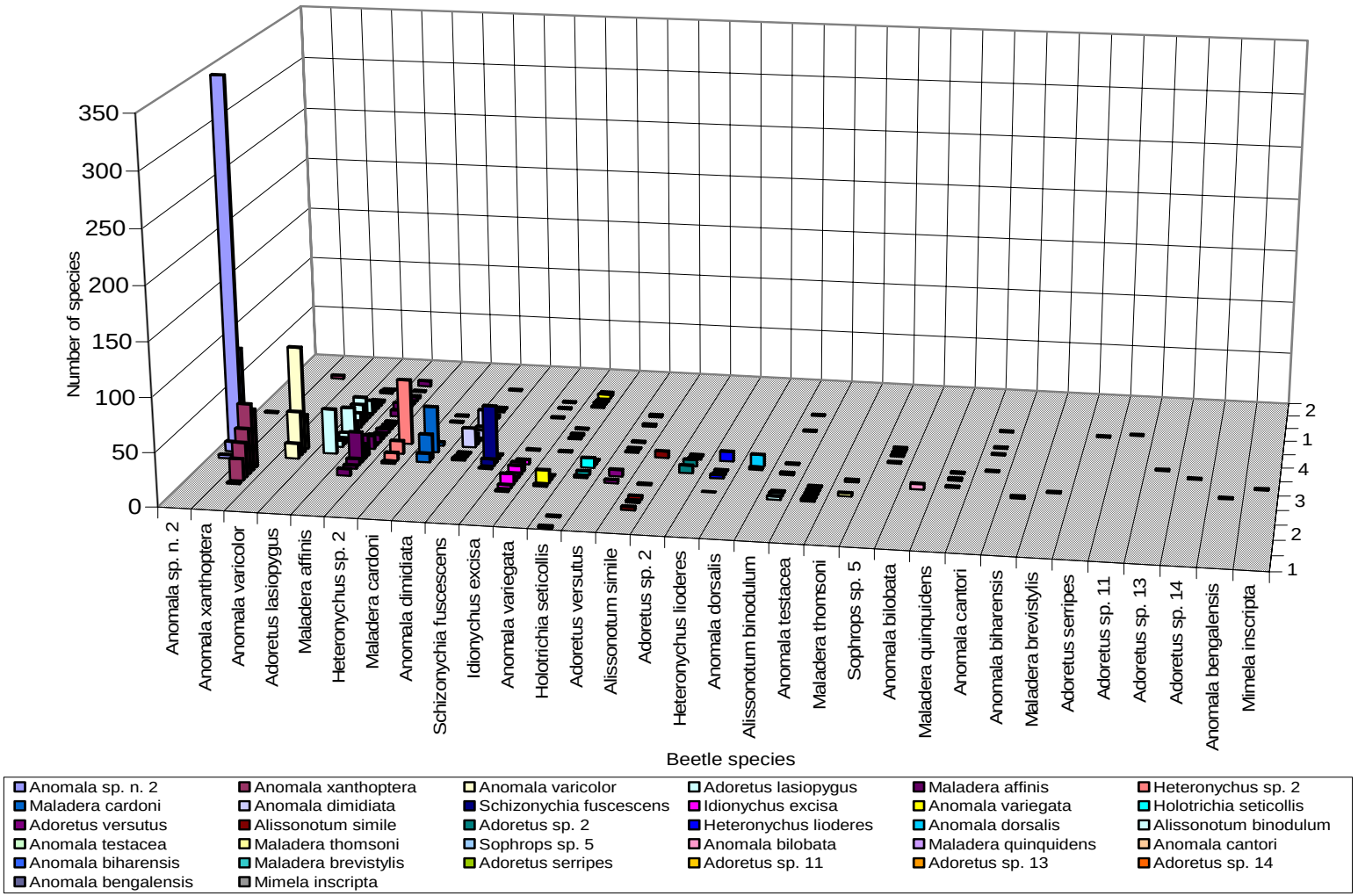



Figure 9.9 Species composition and number of species collected through light traps since January through November 2005 in Gunganagar, Chitwan, Nepal (the number presented in the table are collected over 41 trap nights where light trap was operated 1 night per week).

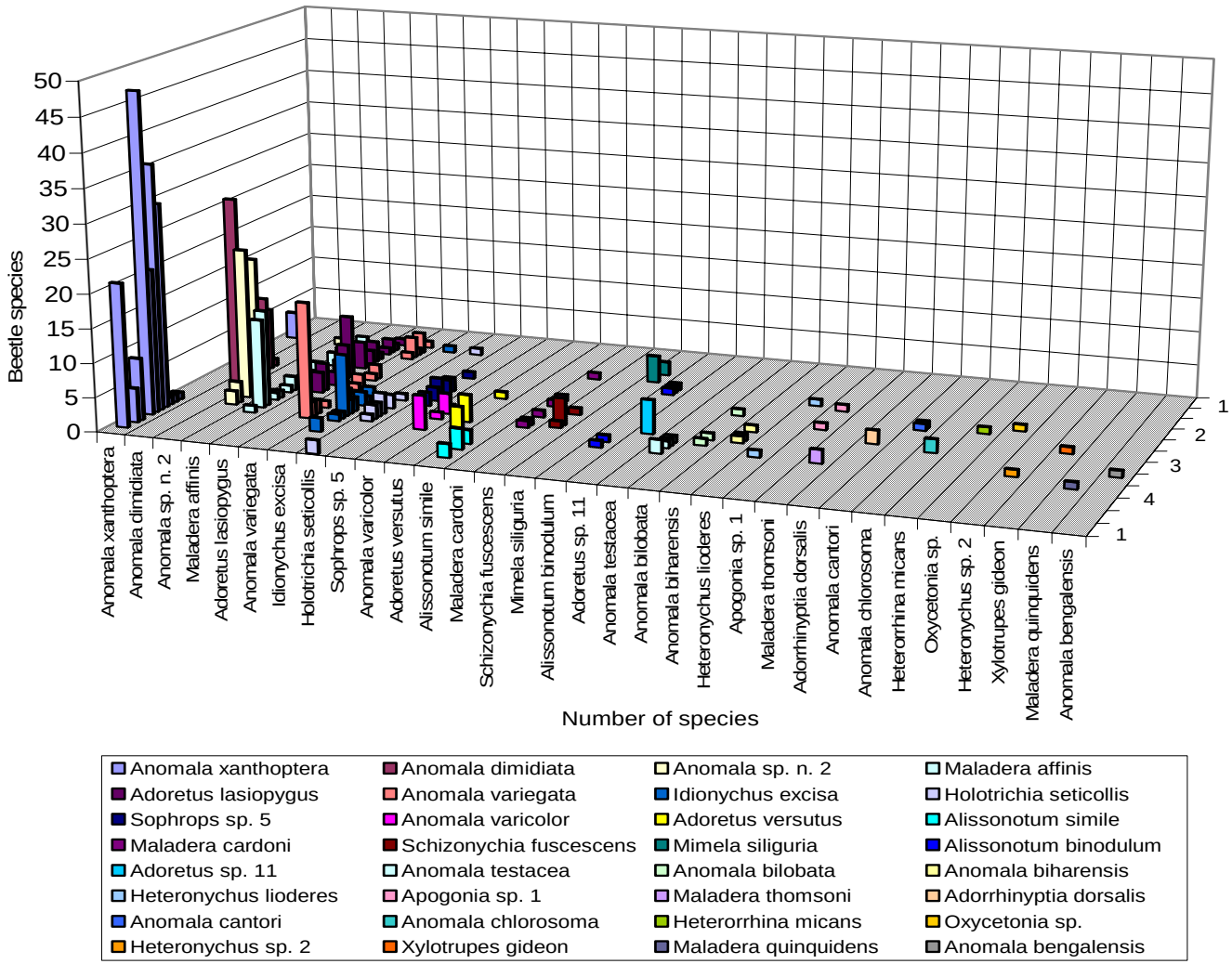



Figure 9.10 Species composition and number of species collected through light traps since January through November 2005 in Gaindakot, Nawalparasi, Nepal (the number presented in the table are collected over 41 trap nights where light trap was operated 1 night per week).

### 9.4.3 Phenology of beetles during 2003-2005

The overall phenology of the adult beetles over the period of two years in two different research sites i.e. in Gunganagar and Gaindakot is presented in is presented in Tables 9.6 and 9.7 respectively.

Table 9.6 Phenology of beetles species observed through light trap collection in Gunganagar, Chitwan, Nepal during November 2003 - November 2005 (the beetle number presented in the table are collected over 96 trap nights where light trap was operated 1 night per week).

| Beetle specimens | Year 2003 | | Year 2004 | | | | | | | | | | | | Year 2005 | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Month | | Month | | | | | | | | | | | | Month | | | | | | | | | | |
| | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| *Ad. Coronatus* Burmeister | | | | | | | | | | | | 1 | | | | | | | | | | | | | 1 |
| *Ad. lasiopygus* Burmeister | 2 | 1 | 1 | 1 | | 637 | 264 | 140 | 8 | 24 | 1 | 7 | 1 | | | | | | 48 | 31 | 65 | 30 | 3 | 3 | 1267 |
| *Adoretus serripes* Arrow | | | | | | 12 | | | | | 4 | | | | | | | | | | | | 1 | | 17 |
| *Adoretus simplex* Sharp | | | | | | 3 | 1 | | | | 2 | 1 | | | | | | | | | | | | | 7 |
| *Adoretus sp.* 10 | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| *Adoretus sp.* 11 | | | | | | | 1 | | | | | | | | | | | | | | | | 1 | | 2 |
| *Adoretus sp.* 13 | | | | | | | | | | | | | | | | | | | | | 1 | | | | 1 |
| *Adoretus sp.* 14 | | | | | | | | | | | | | | | | | | | | 1 | | | | | 1 |
| *Adoretus sp.* 2 | | | | | | 19 | | 2 | | | | | | | | | | | 13 | 2 | | | | | 36 |
| *Adoretus sp.* 9 | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| *Adoretus versutus* Harold | | | | | | 14 | 3 | 6 | 2 | | | 2 | | | | | | 9 | | 2 | 1 | 2 | 2 | | 43 |
| *Adorrhinyptia dorsalis* Burm | | | | | | | 1 | 1 | | | | | | | | | | | | | | | | | 2 |
| *Ali. Binodulum* Fairmaire | | | | | | 1 | | | | | 41 | 3 | | | | | 3 | 4 | 2 | 1 | | 1 | | 1 | 57 |
| *Ali. simile* Arrow | | | | 1 | | 34 | 3 | | | | | 1 | | | | 3 | 5 | 1 | | 6 | | | | | 54 |
| *An. bengalensis* Blanchard | | | 1 | | | 1 | 4 | | | | | | | | | | | | 1 | | | | | | 7 |
| *An. biharensis* Arrow | | | | | | 1 | | | | | | | | | | | | 2 | | | | | | | 3 |
| *An. bilobata* Arrow | | | | | 1 | 50 | 2 | | | | | | | | | | | | 5 | | | | | | 58 |
| *Anomala cantori* Hope | 3 | 7 | | 1 | | 7 | 1 | 8 | 16 | 13 | 1 | | | | | | | | | 1 | 1 | 1 | 1 | | 61 |
| *An. chlorosoma* Arrow | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| *An. comma* Arrow | | | | | 1 | | | | | | | | | | | | | | | | | | | | 1 |
| *An. dimidiata* Hope | 2 | 9 | 2 | 3 | 1 | 17 | 3 | 16 | 32 | 34 | 2 | | | | | | | | 4 | 29 | 28 | 6 | 2 | 1 | 191 |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Anomala dorsalis* | | | | | | | 1 | | | | | | | | | | | 2 | 11 | | | | | | 14 |
| *Anomala euops* Arrow | | | | | | | | | | | 2 | | | | | | | | | | | | | | 2 |
| *Anomala* sp. n. 1 | | | | | | | | | 1 | | | | | | | | | | | | | | | | 1 |
| *Anomala* sp. n. 2 | | | | | | 81 | 12 | 13 | | | | | | | | | 3 | 449 | | | | 1 | | | 559 |
| *An. estacea* Hope | | | | | | | | | | | | | | | | 2 | 4 | | | | | | | | 6 |
| *An. varicolor* Gyllenhal | | | | | | 7 | 3 | 1 | | | | | | | | | 14 | 172 | | | | | | | 197 |
| *An. variegata* Hope | | | | | 1 | 6 | | | | | 2 | 4 | | | | 2 | 14 | | 1 | 3 | 1 | 1 | 10 | | 45 |
| *An. xanthoptera* Blanchard | | | | | 40 | 2 | 63 | | | | | | | | 1 | 156 | 54 | | | | | | | | 316 |
| *Chiloloba acuta* Wiedemann | | | | | | | 1 | | | | | 1 | | | | | | | | | | | | | 2 |
| *Hemiserica nasutella* Ahrens | | | | | | | | | | | 2 | 1 | | | | | | | | | | | | | 3 |
| *Heteronychus liioderes* | | | | | | 69 | 5 | | | | 4 | 1 | | | | | | 5 | 9 | | | | | | 93 |
| *Heteronychus* sp. 2 | | | | | | 3 | | | | | | | | | | | 2 | 19 | 61 | | | | | | 85 |
| *Holotrichia seticollis* Moser | | | | | | 3 | 1 | 1 | | | | | | 1 | 3 | | 5 | 11 | | | | | | | 25 |
| *Idionychus excisa* Arrow | | | | | 2 | 24 | 31 | 1 | | | 1 | 2 | | | | 14 | 24 | 4 | 1 | | 1 | 1 | 1 | | 107 |
| *Maladera affinis* Blanchard | 1 | 4 | | | 1 | 13 | 1 | 4 | 2 | 11 | 59 | 9 | | | | 6 | 37 | 45 | 11 | 6 | 17 | 15 | 6 | | 248 |
| *Maladera brevistylis* Ahrens | | | | | | | | | | | | | | | | | | 1 | | | | | | | 1 |
| *Maladera cardoni* Brenske | | | | | | 51 | 6 | 12 | | | | | | | | | | 74 | 3 | | 2 | | | | 148 |
| *Maladera quinquidens* Brenske | | | | | | | | | | | | | | | | | | 3 | 1 | | | | | | 4 |
| *Maladera thomsoni* Brenske | | | | | | | | | | | | | | | | | 3 | 2 | | | | | | | 5 |
| *Mim.cf. fulgidivittata* Blanchard | | | | | | | | | | 1 | | | | | | | | | | | | | | | 1 |
| *Mim. inscripta* Nonfried | | | | | | | | | | | | | | | | | | | 1 | | | | | | 1 |
| *Mimela siliguria* Arrow | | | | | | | | | | | 3 | 6 | | | | | | | | | | | | | 9 |
| *Mimela* sp. n | | | | | | 1 | | 1 | | | 1 | 2 | 1 | | | | | | | | | | | | 6 |
| *Oxycetonia histro* Oliver | | | | 1 | | 2 | | | | | | | | | | | | | | | | | | | 3 |
| *Oxycetonia variicolor* F. | | | | 1 | | | | | | | | | | | | | | | | | | | | | 1 |
| *Parastasia rufopicta* Westwood | | | | | | | 1 | | | | | | | | | | | | | | | | | | 1 |
| *Pentodon algerinum indicum* Endroedi | | | | | | 1 | | | | 1 | | | | | | | | | | | | | | | 2 |
| *Popillia birmanica* Arrow | | | | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | 4 |
| *Rhamphadoretus suillus* Arrow | | | | | | | 2 | | | | 2 | | | | | | | | | | | | | | 4 |
| *Schizonychia fuscescens* Blanchard | | | | | | 6 | 4 | 2 | | | | | | | | | 1 | 61 | | | | | | | 74 |
| *Sophrops* spec. 1 | | 1 | | | | 1 | 1 | 2 | 3 | | | | | | | | | | | | | | | | 8 |
| *Sophrops* spec. 5 | | | | | | | 2 | | | | | | | | | | | | 1 | 4 | | | | | 7 |
| **Number of individuals** | **8** | **22** | **4** | **9** | **48** | **1070** | **418** | **210** | **64** | **84** | **127** | **41** | **2** | **1** | **3** | **4** | **188** | **177** | **921** | **173** | **109** | **62** | **27** | **22** | **3794** |

Table 9.7 Phenology of beetles species observed through light trap collection in Gaindakot, Nawalparasi, Nepal during November 2003 - November 2005 (the beetle number presented in the table are collected over 96 trap nights where light trap was operated 1 night per week).

| Beetle specimens | Year 2003 | | Year 2004 | | | | | | | | | | | | Year 2005 | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Month | | Month | | | | | | | | | | | | Month | | | | | | | | | | |
| | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| *Adoretus lasiopygus* Burmeister | 1 | | | | 3 | 1 | 2 | | | 1 | | 1 | | | | | | | | 9 | 17 | 4 | 6 | 4 | **49** |
| *Adoretus simplex* Sharp | | | | | | | | | | | | | 1 | | | | | | | | | | | | **1** |
| *Adoretus* sp. 11 | | | | | | | | | | | | | | | | | | | 5 | | | | | | **5** |
| *Adoretus versutus* Harold | | | | | | | | | | 2 | | | 1 | | | | | 7 | | | 1 | | | | **11** |
| *Adorrhinyptia dorsalis* Burm. | | | | | | | | | | | | | | | | | | | | 2 | | | | | **2** |
| *Ali. binodulum* Fairmaire | | | | | | 1 | | 2 | | | 1 | | 1 | | | | 1 | 1 | | | | 1 | 2 | | **10** |
| *Alissonotum simile* Arrow | 3 | 1 | | 4 | 2 | 1 | 2 | 3 | 3 | 2 | | 1 | 1 | | 2 | 3 | 2 | | | | | | | | **30** |
| *Anomala bengalensis* Blanchard, 1851 | | | | | | | 1 | | | | | | | | | | | | 1 | | | | | | **2** |
| *Anomala bilobata* Arrow | | | | | | 2 | 5 | | | | | | | | | | | 1 | 1 | | 1 | | | | **10** |
| *Anomala cantori* Hope | | | | | 3 | | 2 | 6 | | 2 | 1 | | | | | | | | | | 1 | 1 | | | **16** |
| *Anomala cf. biharensis* Arrow | | | | | | | | | | | | | | | | | | | 2 | 1 | | | | | **3** |
| *Anomala chlorosoma* Arrow | | | | | | | | | | | | | | | | | | | | 2 | | | | | **2** |
| *Anomala comma* Arrow | | | | | | | | | | | | | 1 | | | | | | | | | | | | **1** |
| *Anomala dimidiata* Hope | | 1 | | 1 | 4 | 1 | 7 | 5 | | 3 | 1 | | | | | | | | | 31 | 23 | 1 | | | **78** |
| *Anomala* sp. n. 2 | | | | | | | | | | | | | 1 | | | | | 5 | 48 | | | | | 1 | **55** |
| *Anomala* sp. n. 3 | | | | | | | | | 1 | | | | | | | | | | | | | | | | **1** |
| *Anomala testacea* Hope | | | | | | | | | | | | | | | | | 2 | 3 | | | | | | | **5** |
| *Anomala varicolor* Gyllenhal | | | | | | 1 | | | | | | | | | | | 5 | 1 | 4 | | | | | | **11** |
| *Anomala variegata* Hope | | | | | | 1 | | | | | | | 6 | | | | 17 | 5 | | 4 | 3 | | 1 | 7 | **44** |
| *Anomala xanthoptera* Blanchard | | | | | 3 | | | | | | | | | | 21 | 61 | 88 | 2 | | | | | | 4 | **179** |
| *Apogonia* sp. 1 | | | | | | | | | | | | | | | | | | | | | 1 | 1 | | | **2** |
| *Heteronychus lioderes* | | | | 1 | | | 2 | 1 | | | | | 1 | | | | | 1 | | | | | 1 | | **7** |
| *Heteronychus* sp. 2 | | | | | | 1 | 1 | | 1 | 1 | 2 | 1 | | | | | | 1 | | | | | | | **8** |
| *Heterorrhina micans* | | | | | | | | | | | | | | | | | | | | | | 1 | | | **1** |
| *Holotrichia seticollis* Moser | | | | | | 1 | | | | | | | | | 2 | | | 8 | 2 | 1 | | | | 1 | **15** |
| *Idionychus excisa* Arrow | | | | | | | | | | | | | | | | 2 | 1 | 14 | 5 | | | | | 1 | **23** |
| *Maladera affinis* Blanchard | 1 | | | | 2 | | 9 | 1 | 7 | 19 | 1 | | 1 | | | | 1 | 27 | 3 | 3 | 3 | 2 | 4 | 3 | **87** |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Maladera cardoni* Brenske | | | | | | 1 | 1 | 1 | | | | | | | | | | 1 | 2 | 1 | 1 | | 1 | | **9** |
| *Maladera quinquidens* Brenske | | | | | | | | | | | | | | | | | | 1 | | | | | | | **1** |
| *Maladera schenklingi* Moser | | | | | | | | 1 | | | | | | | | | | | | | | | | | **1** |
| *Maladera thomsoni* Brenske | | | | | | | | | | | | | | | | | | 2 | | | | | | | **2** |
| *Mimela siliguria* Arrow | | | | | | | | | | 4 | 1 | | | | | | | | | | | | 4 | 2 | **11** |
| *Oxycetonia* sp. | | | | | | | | | | | | | | | | | | | | | | 1 | | | **1** |
| *Schizonychia fuscescens* Blanchard | | | | | | 2 | | | | | | | | | | | | | 5 | 1 | | | | | **8** |
| *Sophrops* spec. 1 | | | | 1 | | | | 2 | | | | | | | | | | | | | | | | | **3** |
| *Sophrops* spec. 5 | | | | | | | | | | | | | | | | | | | 2 | 5 | 4 | 1 | | | **12** |
| *Xylotrupes gideon* L. | | | | | | | | | | | 2 | | | | | | | | | | 1 | | | | **3** |
| **Number of individuals** | **5** | **2** | **0** | **7** | **17** | **13** | **32** | **22** | **12** | **32** | **10** | **4** | **14** | **0** | **25** | **66** | **117** | **80** | **80** | **60** | **56** | **13** | **19** | **23** | **709** |

The diversity of the beetle species (Ahrens, 2005a & Ahrens, 2005b) that were recorded from light traps during the period of two years (November 2003 to October 2005) with their virtual figures is presented in the appendix 9.1.

9.4.3.1 The seasonal abundance of beetle specimens during 2003-2005

The seasonal abundance of the adult beetles in terms of total number of specimens over the period of two year (2003-2005) in Gunganagar and Gaindakot research sites is presented in Figure 9.11. This study has convincingly demonstrated the involvement of the highest number of beetle specimens during the month of May (nearly 1100 out of 4 trap nights) followed by April and late September, whereas, the least numbers are recorded during December and January. Similar pattern of adult flight has been recorded in both the sites; however, the total number of beetle catches is very low in Gaindakot (nearby forest site) as compared to Gunganagar (crop site). More number of beetle specimens was recovered in the former site compared to later. From these experiments, it is evident that the beetle flight occurred in two different seasons of the year one large peak in May followed by another small peak in the month of September to October. In general, the adult beetle becomes active onwards March and reaches to its peak during May and activity almost ceased by the end of November.

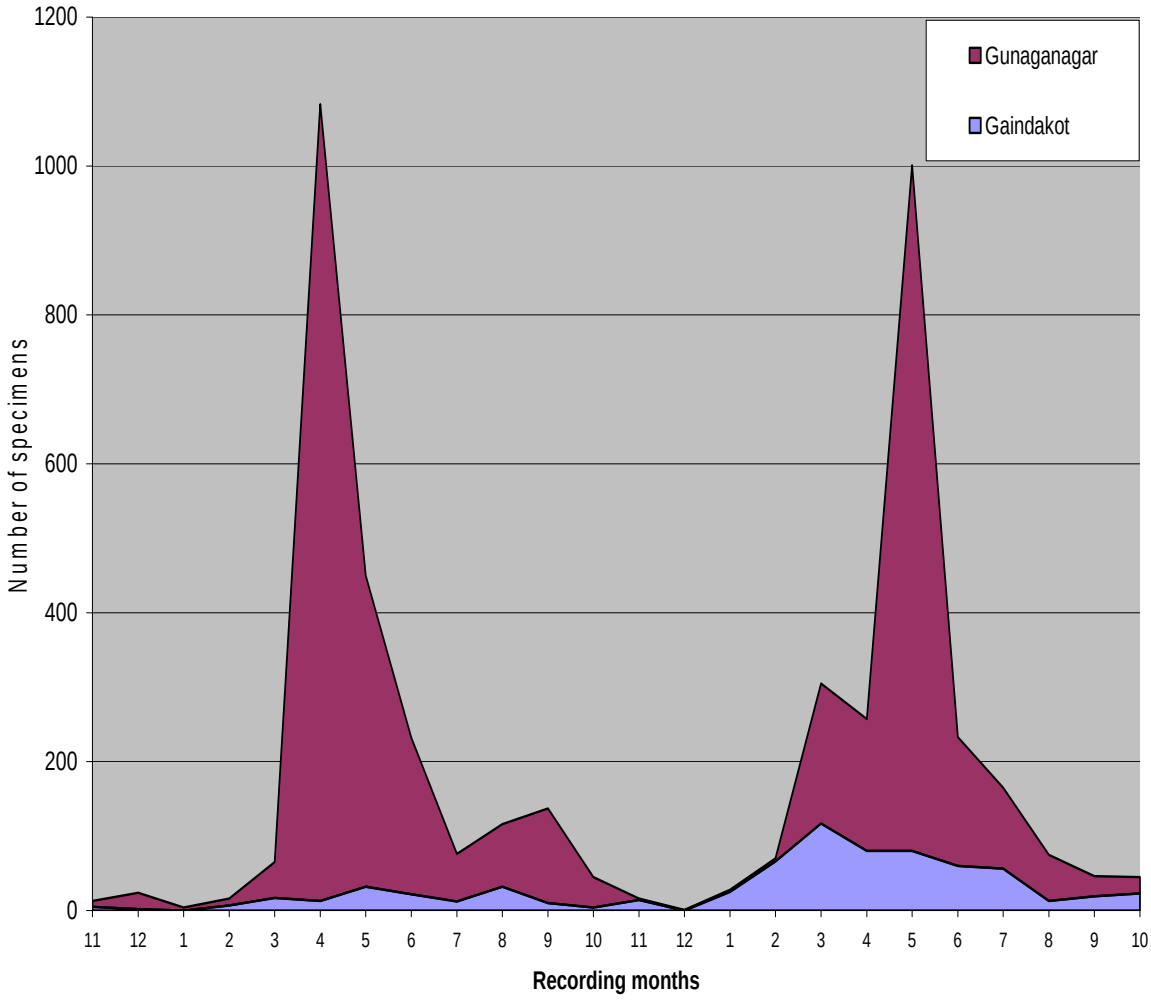



Figure 9.11 Seasonal abundance of beetle specimens during 2003-2005 in Gunganagar and Gaindakot research sites in Nepal (96 trap nights based on 4 trap nights x 24 months).

9.4.3.2 The seasonal abundance of beetle species during 2003-2005

The overall species recorded in both the research sites over the study period of two years (November 2003 through October 2005) is presented in Figure 9.12. This study has convincingly demonstrated that the large number of species involvement in Nepalese agricultural fields. In general, more than ninety different beetle species were found to be involved over the study period. Very interestingly, more than forty different species were found to be involved during the month of May in Gunanagar research sites, whereas, three times less number of species in Gaindakot research site.

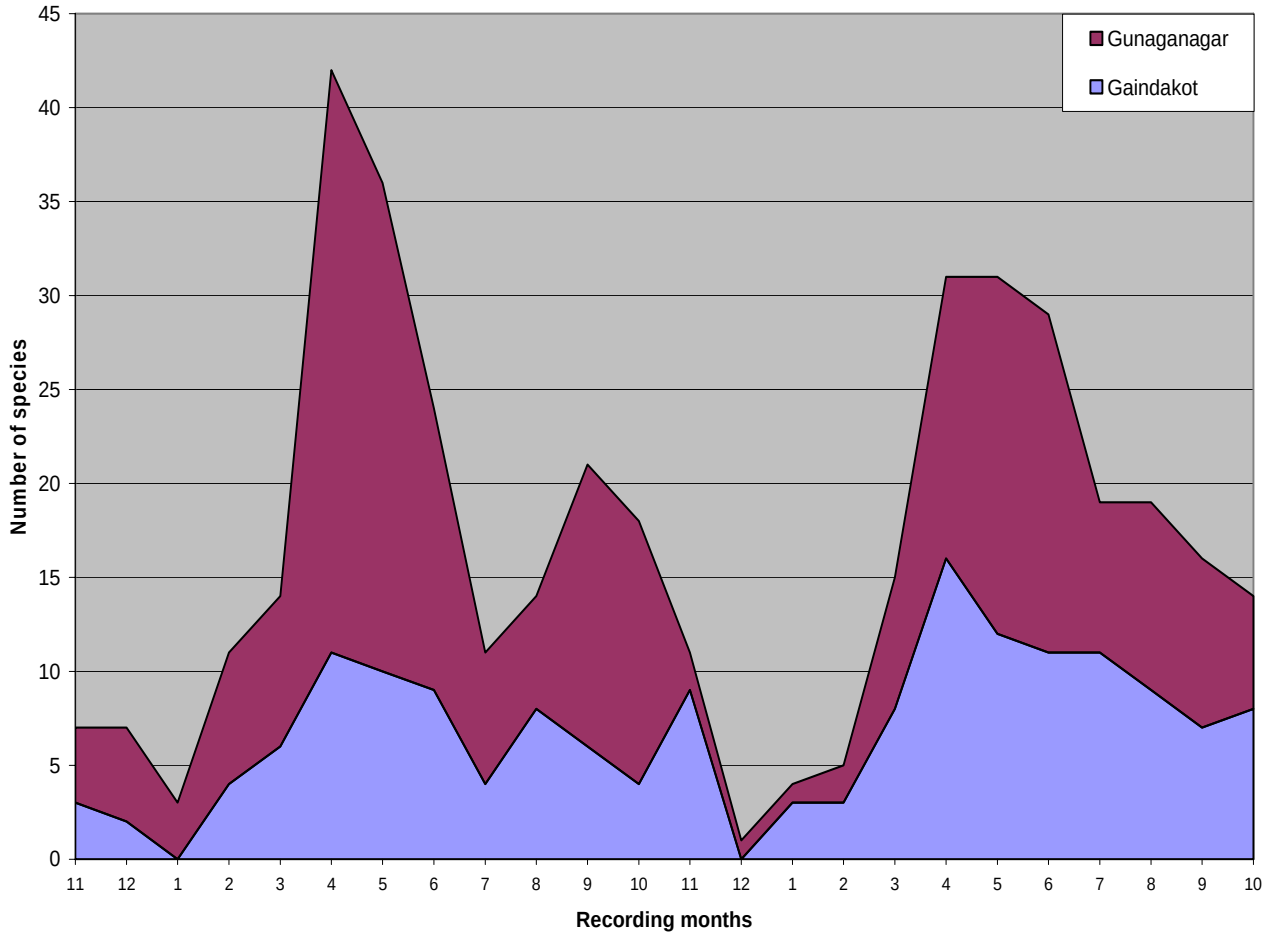



Figure 9.12 Seasonal abundance of beetle species in Gunganagar and Gaindakot research sites over the period of 2003-2005 in Nepal (96 trap nights based on 4 trap nights x 24 months).

## 9.5 DISCUSSION

Various species of beetle species are involved in the various agro-ecological sites. The possible reasons of variation of the species could be due to variation in the altitudes, vegetation, crop grown, soil and other characteristics etc. Similarly, fluctuation of beetle species in two different seasons mainly in low tropical belts (terai) might be due to variation in species, short duration of life cycle and overlapping generation in the same year. Unlike low altitude sites, flight of adult beetle does not reach into two peaks in the same year in mid hills area. This could be the reason of larval

occurrence only once a year or sometimes in alternate year. Similarly, various species of beetles are abundant in terai areas compared to mid hills. The possible reason could be due to diverse range of crops and favourable temperature for the breeding for adult beetles and species involvement.

Adults of white grubs attracted to the light trap with large body size were generally intact and easy to identify, however, it was found difficult in case of beetles that are having smaller body size. The former type of beetles was abundantly found in March but in a decreasing trend in winter months. The emergence of beetle at the terminal period of February may have resulted from the start of warm weather after the winter. The beetles lay eggs in the soil and larvae (grubs) emerge and begin to attack the host crop throughout early monsoon and again reappear in early winter. In both the sites, potato and other vegetables are relayed after maize therefore, if such crop harvesting is delayed, greater extent of losses can occur in vegetable production. Similarly, attack in the maize crop can result into retarded growth or mortality of the plant. The catches in general were found decreasing along with the monsoon and high temperature. There is some indication that early season (April-June) temperature and rainfall may determine survival of adult beetles, however, higher temperature as well as heavy rain is detrimental for adult flights. A general correlation existed between the compositions of beetles' catches and the seasons in both the study area. Generally, the beetles that are having larger body size, occur at low rainfall period which can also induce the considerable extent of damages, whereas, fewer damages and very low catches are resulted when the rainfall remains heaviest.

The present findings with regard to species prevalence suggest the involvement of large number of phototropic insect in the cropping area as compared to river basin area. The possible reason for this may be the availability of appropriate hosts in the former site compared to later site. Further more, more number of photoinsensitive beetles mainly water beetles and few other species may have abundant in the later sites. In general, the seasonal abundance of the beetle species largely suggests the peak occurrence as well as lean period of beetle activity which may be taken as indication of possible damage in the following cropping seasons.

## 9.6 CONCLUSIONS

White grubs are nationally important pest insect in Nepal. Monitoring of these pests has provided some basic information of their phenology and pest dynamics. Results of the monitoring studies were also found corresponding with previous findings of the experiments conducted by Pandey *et al.* (1993) in Lumle conditions. The beetles monitoring by these traps could reduce the abundance of the adults to some extent. Monitoring should be conducted in a larger area in collaboration with District Agriculture Development Offices at the sites where the problems have been reported. Monitoring studies are effective tools for generating basic information on the variation in pest distribution and their seasonal fluctuation with regard to effective pest control.

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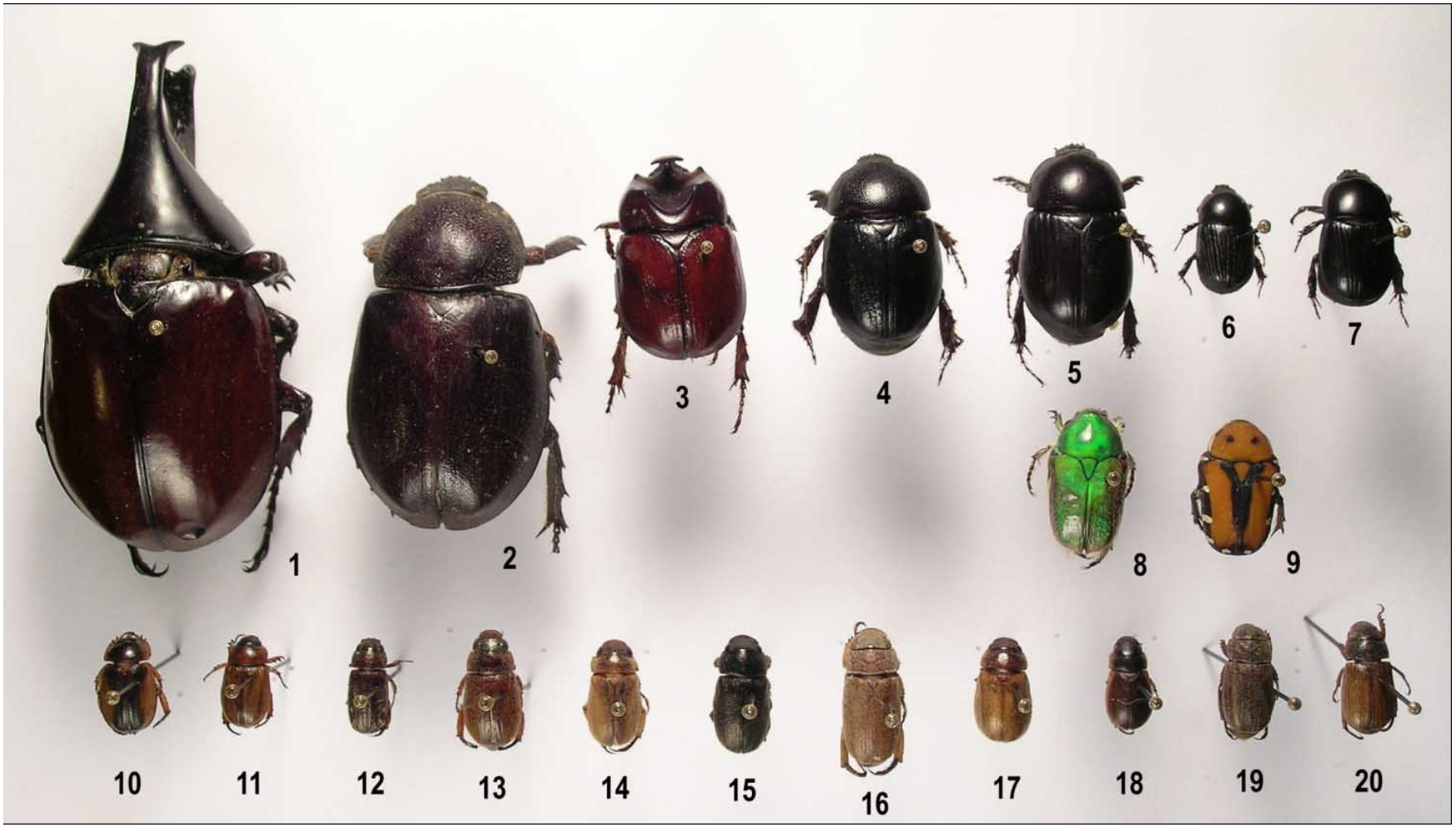
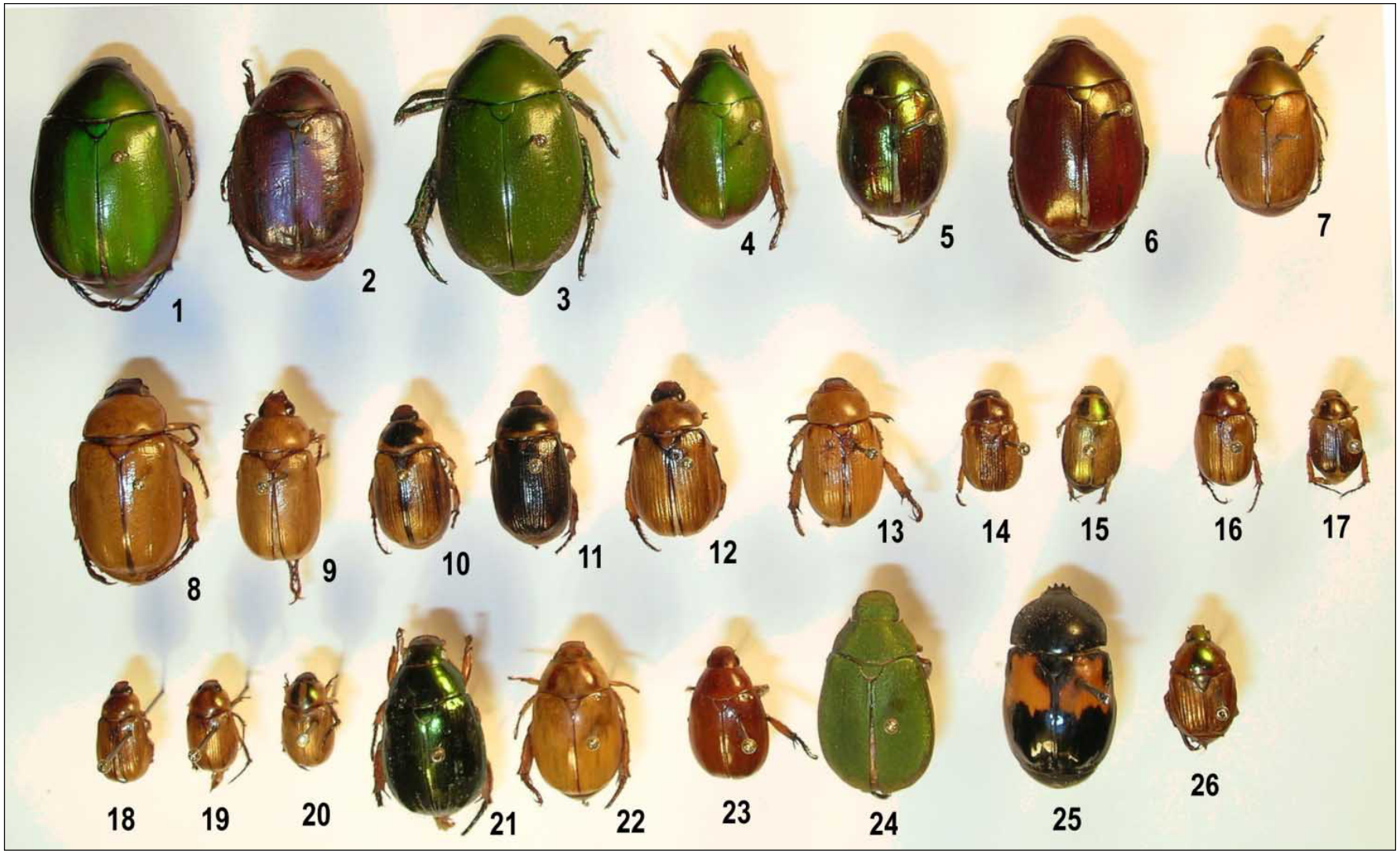
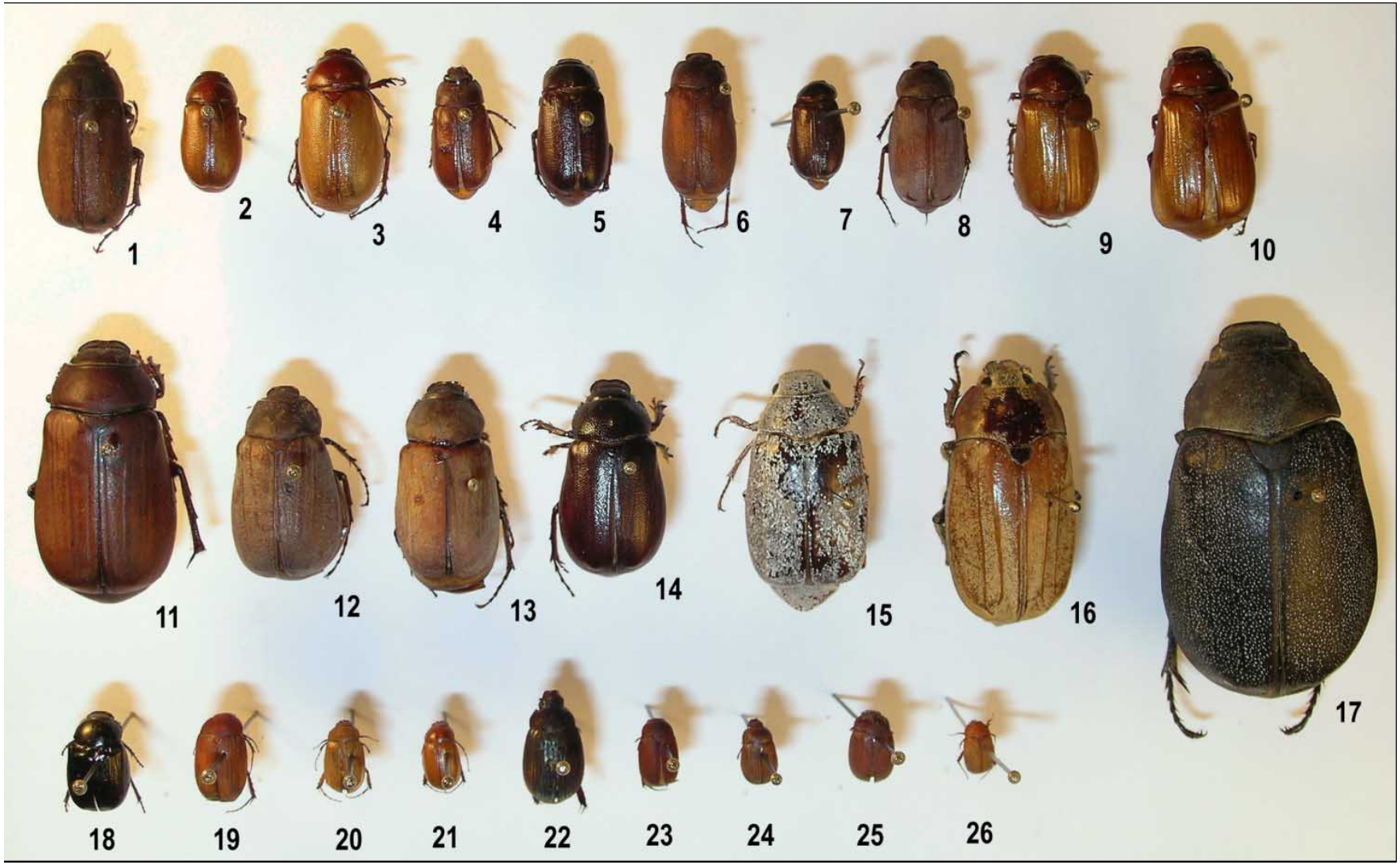
**Appendix 9.1 Diversity of Scarabaeid beetles as collected from light traps in some farming sites of Nepal**




**1:** *Xylotrupes gideon* L. (male); **2:** *Xylotrupes gideon* L. (female); **3:** *Phyllognathus dionysius* F.; **4:** *Pentodon algerinum indicum* Endroedi; **5:***Alissonotum binodulum* Fairmaire; **6:** *Alissonotum simile* Arrow; **7:** *Heteronychus lioderes* Redtenbacher; **8:** *Chiloloba acuta* Wiedemann; **9:***Oxycetonia histro* (Oliver); **10:** *Adorrhinyptia dorsalis* Burmeister; **11:** *Rhamphadoretus suillus* Arrow; **12:** *Adoretus coronatus* Burmeister; **13:***Adoretus versutus* Harold; **14:** *Adoretus lasiopygus* Burmeister; **15:** *Adoretus serratipes* Arrow; **16:** *Adoretus simplex* Sharp; **17:** *Adoretus* sp. 2;




**1:** *Anomala dimidiata* (Hope); **2:** *Anomala dimidiata* (Hope) (colour variation); **3:** *Anomala chlorosoma* Arrow; **4:** *Anomala perplexa* Hope; **5:** *Mimela fulgidivittata* Blanchard; **6:** *Anomala cantori* (Hope); **7:** *Anomala xanthoptera* Blanchard; **8:** *Anomala bengalensis* Blanchard; **9:** *Anomala bilobata* Arrow; **10:** *Anomala varicolor* (Gyllenhal); **11:** *Anomala varicolor* (Gyllenhal) (colour variation); **12:** *Anomala marginipennis* Arrow; **13:** *Anomala biharensis* Arrow; **14:** *Anomala* sp. n. 1; **15:** *Anomala comma* Arrow; **16:** *Anomala euops* Arrow; **17:** *Anomala variegata* Hope; **18:** *Anomala* sp. n. 2; **19:** *Mimela siliguria* Arrow; **20:** *Anomala* sp. n. 3; **21:** *Mimela horsfieldii* Hope; **22:** *Mimela inscripta* (Nonfried); **23:** *Mimela bicolor* Hope; **24:** *Mimela sericea* Ohaus; **25:** *Parastasia rufopicta* Westwood; **26:** *Popillia birmanica* Arrow.




**1:** *Asactopholis dehradunensis* Mittal; **2:** *Schizonychia fuscescens* Blanchard; **3:** *Idionychus excisa* Arrow; **4:** *Sophrops* sp. 5; **5:** *Sophrops* sp. 1; **6:** *Sophrops* sp. 2; **7:** *Sophrops cardoni* Brenske; **8:** *Sophrops* sp. 3; **9:** *Holotrichia nigricollis* Brenske; **10:** *Holotrichia* sp. 2; **11:** *Holotrichia sikkimensis* Brenske; **12:** *Holotrichia pruinosa* Wiedemann; **13:** *Holotrichia seticollis* Moser; **14:** *Holotrichia anthracina* Brenske; **15:** *Cyphochilus pygidialis* Nonfried; **16:** *Melolontha indica* Hope; **17:** *Lepidiota albistigma* Burmeister; **18:** *Apogonia* sp. 1; **19:** *Maladera affinis* (Blanchard); **20:** *Maladera thomsoni* (Brenske); **21:** *Maladera cardoni* (Brenske); **22:** *Maladera iridescens* (Blanchard); **23:** *Maladera schenklingi* (Moser); **24:** *Maladera quinquidens* (Brenske); **25:** *Maladera pokharae* Ahrens; **26:** *Hemiserica nasutella* Ahrens.

# Chapter 10

## Life tables and life cycles studies of the common species of white grub in some agro-ecological zones of Nepal

# Chapter 10

# Life tables and life cycles studies of the common species of white grub in some agro-ecological zones of Nepal

## 10.1 SUMMARY

In order to understand the natural mortality factors associated in regulating insect population, life table studies and life cycle studies were conducted in 2003/04 with the beetle larvae of three different agro-environments. These study sites represented with low hill area, low mid hill area and mid hill area of Chitwan, Tanahun and Parbat Districts respectively of Nepal. Along with the life table studies, the life cycle of the similar kinds of white grub's species was undertaken in the subsequent seasons. The major objectives of these studies were to know the disturbing factors of white grubs and duration of insect stages so as to plan the microbial control programs in such areas. Numerous kinds of natural antagonists coupled with environmental stresses were recorded in suppressing the larval stages. The life table revealed numerous kinds of natural antagonists (*Metarhizium anisopliae* and *Beauveria bassiana*) and endoparasitic nematode (Mermithidae) coupled with environmental stresses (physical factors) were recorded in suppressing the larval stages. In the same way, different duration of life cycle was observed with different species of beetles involved in different agro-ecological zones. The larval duration of *Lepidiota albistigma* Burmeister, was found significantly longer (284 days), followed by *Maladera affinis* Blanchard (58 days) and *Xylotrupes gideon* L. (57 days) and this parameter was found significantly difference ($p<0.001$) among the species. The basic information obtained from the studies would be relevant in planning the microbial control of white grubs. White grubs can be controlled in a timely and economical manner if proper controls are applied in the right time.

## 10.2 INTRODUCTION

Different species of white grubs are involved in different crops in Nepal (Neupane, 1995). It is suspected that they have different duration of life cycle and damage occurs sometimes every year whereas, in another case in alternate year. Yadava and Vijayvergia (1994), reported that some species of scarabs complete their life cycle in as little as one-year, the most important and damaging species take three years for each generation. Thus damage is often cyclical, with most damage occurring every third year. In general, one year life cycle is common in beetles of nearly all groups and in such species two or more winters (or dry seasons) is passed in the larval stage, while pupae, adults, eggs and first instars larvae occur at fixed seasons within a single year so that the advantages of seasonal adaptations are retained to a considerable extent. In beetles with one generation per year (univoltine) diapause is most common in the adult stage (Crowson, 1975) less so in the larvae, and comparatively rare in eggs or pupae. The factors which initiate or trigger it may be day-length changes in the higher latitudes, changes in temperature or humidity, or possibly sometimes changes affecting the food whereas, hibernation may be induced by decreasing the day length. Crowson (1981) further reported in univoltine species subject to heavy attack by more or less specific parasites, marked population cycles, often with periods of the order of 4 or 5 years, are liable to occur in natural populations. Multivoltine species

(with two or more generations per year), are less spread in Coleoptera in seasonal climates. The life cycle of white grubs involved in Nepalese farming are not studied yet, therefore, this study was carried out to know the life cycle and mortality factors (life tables) of most common species of white grubs of the fungus application experiments.

The population of insect pests never remains constant for long because the carrying capacity of the environment imposes a check over it (Trehan and Butani, 1949). There are many natural mortality factors, such as, biotic and abiotic factors, however, their role in pest dynamics is seldom considered. Many scientists have studied the mortality factors and described in different ways. Among those as presented by Harcourt (1969) on Colorado beetle in Ontario, Canada is remarkable. He described the causes of mortality categorically such as no egg deposition by the insects or may be the eggs are infertile eggs or due to rainfall or cannibalism or predation, rainfall starvation, parasite, summer adults, emigration, frost damage etc. He sampled the developmental stages of the beetle on a regular basis and the data show a decline in numbers through the generation due to various disturbing factors such as density independent and density dependent factors.

Life tables are quantitative descriptions of the life history of organisms relative to age or growth stage. Life tables include consideration of survival from one age (or stage) to the next, and the reproduction that occurs at each age (or stage). It also gives an age-specific summary of the mortality rates operating on a population. It is used to study insect population at a specific time and space. To construct a life table the mortality during and between the different ages (or stage) is determined which then permits derivation of survivorship curves. This information can be used to predict possible rates of population change, and can aid in identification of mortality factors operating at different ages (or stages) in the life cycle. Amongst the mortality factors, the role of abiotic factor such as natural mortality through antagonists has been considered in this study. Mortality factors based on sampling eggs, larval stages, and pupae, were established in three different agro-ecological zones of Nepal, where augmentative release of fungus has been planned.

## 10.3 MATERIAL AND METHODS

### 10.3.1 Life table studies

The study was carried out in four different sites of Nepal, which includes, Gunganagar, Nahala and Pang in Chitwan, Tanahun and Parbat Districts respectively of Nepal. Initially, a total of 500 first larvae of white grubs were collected from the fields of each site since the April through May of 2004. After collection, they were further grouped for making uniform species under microscope at the insect pathology laboratory, IAAS, Rampur. Rearing was carried out individually into the rearing poly pots (4.5 cm diameter and 6 cm height) in the favorable soil moisture at 22 $^{o}$C room temperature. Slices of potatoes were supplied as artificial diets as and when necessary. The observation parameter of this study includes the successive life stages over the collected larvae in relation to natural mortality and expectancy of the insect's life for three generations. The adult insects were identified later by the reference collection established by Prof. Dr Peter Nagel and Dr Dirk Ahrens. Interpretation was done mainly on the mortality factors and population growth with following abbreviated forms.

The abbreviations are expressed as;

x = Pivotal age class
lx = Number surviving at the start of age interval x
dx = Number dying during the age interval x to x +1
qx = Rate of mortality during the age interval x to x +1
Lx = Average of number of individuals between age x to x +1 (lx + lx =1)/2
Tx = Total number of individuals at age x and beyond (all Lx from bottom)
Ex = Life expectancy (Tx/lx)

### 10.3.2 Life cycle studies

In order to know the life cycle of very common and most damaging species of white grubs, this study was performed in the same sites where the fungus application was carried out. These sites include Gunganagar (Chitwan), Nahala (Tanahun) and Pang (Parbat) Nepal. Initially, all kinds of larvae obtained during digging were collected from the damaging sites in June-July of 2004. After collection, only the dominant and abundant species were taken into the lab and further grouped based on morphology, larval characteristics and instars for further study. Based on morphology, they were grouped into small, medium and large larvae with a batch of 200 each. Head capsule was considered to differentiate them into different larval instars. Larvae were reared individually into a poly vials (4.5 cm diameter 6 cm height) with the supplement of artificial diets such as slices of potato until the completion of their life cycle at 22-23 $^{o}$C. Soil moisture inside the poly vial was maintained not less than 17% so as to avoid the stress situation. The reason of larval death was explained and adults were preserved into 99% alcohol into the same vials for further identification.

Observation parameters include quantitative as well as qualitative information. The first kind of information includes the larval body sizes (small, medium and large) irrespective of the instars, movement (slow and fast), locomotion (dorsal, ventral and dorso-ventral) and aggressiveness (aggressive and less aggressiveness) of the larvae. Similarly, the total duration of insect stages is taken as quantitative information and insect species involved in particular sites were later identified based on the reference collection and with the key provided by Prof. Dr Peter Nagel and Dr Dirk Ahrens during their monitoring visits in Nepal.

### 10.3.3 Activity of common beetle species in the research sites

In addition to the life cycle and life table studies in laboratory, the activities of the common beetle species were also studied in farmer's field. This was carried out in Gunganagar (Chitwan), Nahala (Tanahun) and Pang (Parbat). In every site, a total of four farmer's fields were selected and the sampling was carried out within 1 m$^2$ area from October 2004-October 2005. The different stages of the beetles (egg, larva, pupa and adult) recovered during the digging were collected and their occurrences were recorded. Their activities in terms of occurrence at different stages and crop damages are described.

## 10.4 RESULTS

### 10.4.1 Life table studies

From the experiments, it is evident that the considerable number of mortality has been recorded in the early instars larvae followed by advanced stages. Conversely, low mortality is observed in adult stages compared to initial life stages. This situation happened more or less to all the life stages of the insects of all the study sites. The possible reasons might be due to unsuitable environment such as soil moisture and temperature coupled with the artificial food. In general, the result has shown that the life expectancy is considerably short during early instars larvae compared to advance stages. The findings of the life table study of three different research sites of Nepal are presented in Tables 10.1-10.3.

Table 10.1 Life table of *Maladera affinis* Blanchard under laboratory condition (22-23 $^{o}$C) of IAAS, Rampur, Chitwan, Nepal, 2004 (larvae collected from Gunganagar, Chitwan and observed for 87 days).

| Age (x) | Surviving at start (1x) | Number dying (dx) | Mort. rate (qx) | Mortality factors | No. bet. age x and next (Lx) | No. beyond age x (Tx) | Expectancy of life (ex) |
|---|---|---|---|---|---|---|---|
| First instars | 200 | 98 | 49.00 | Infertility and unknown factors | 151.00 | 314.00 | 1.57 |
| Second instars | 102 | 49 | 48.04 | Unknown factors, Physical, fungus (5), Mermithidae (2) | 77.50 | 163.00 | 1.59 |
| Third instars | 53 | 19 | 35.85 | Unknown factors, insect fungus (8) | 43.50 | 85.50 | 1.63 |
| Pupae | 34 | 9 | 26 | Unknown factors, physical | 29.50 | 42.00 | 1.23 |
| Adults | 25 | 2 | 8 | Unknown factors, physical | 12.50 | 12.50 | 0.50 |

Table 10.2 Life table of *Xylotrupes gideon* L. under laboratory condition (22-23 $^{o}$C) of IAAS, Rampur, Chitwan, Nepal, 2004 (larvae collected from Nahala, Tanahun and observed for 85 days).

| Age (x) | Surviving at start (1x) | Number dying (dx) | Mort. rate (qx) | Mortality factors | No. bet. age x and next (Lx) | No. beyond age x (Tx) | Expectancy of life (ex) |
|---|---|---|---|---|---|---|---|
| First instars | 200 | 86 | 43.00 | Infertility and unknown factors | 107.00 | 308.00 | 1.54 |
| Second instars | 114 | 48 | 42.11 | Unknown factors, insect fungus (4), Mermithidae (1) | 90.00 | 201.00 | 1.76 |
| Third instars | 66 | 25 | 37.88 | Unknown factors, physical, insect fungus (9) | 53.50 | 111.00 | 1.68 |
| Pupae | 41 | 4 | 9.76 | Unknown factors, physical | 39.00 | 57.50 | 1.41 |
| Adults | 37 | 3 | 8.11 | Unknown factors | 18.50 | 18.50 | 0.50 |

Table 10.3 Life table of *Lepidiota albistigma* Burmeister under laboratory condition (22-23 $^{o}$C) of IAAS, Rampur, Chitwan, Nepal, 2004 (larvae collected from Pang, Parbat and observed for 325 days).

| Age (x) | Surviving at start (1x) | Number dying (dx) | Mortality rate (qx) | Mortality factors | No. bet. age x and next (Lx) | No. beyond age x (Tx) | Expectancy of life (ex) |
|---|---|---|---|---|---|---|---|
| First instars | 200 | 68 | 34.00 | Physical, Unknown, fungus (3) | 116.00 | 392.00 | 1.96 |
| Second instars | 132 | 40 | 30.31 | Physical, unknown, fungus (2), Mermithidae (1) | 112.00 | 276.00 | 2.09 |
| Third instars | 92 | 26 | 28.27 | Physical, Unknown, insect fungus (6) | 79.00 | 164.00 | 1.79 |
| Pupae | 66 | 14 | 21.21 | Unknown factors | 59.00 | 85.00 | 1.29 |
| Adults | 52 | 6 | 11.53 | Unknown factors | 26.00 | 26.00 | 0.50 |

The study of the mortality factors have clearly shown that the no definite trend of the occurrence of insect fungus has been associated in these study, however, more grubs were succumbed with fungal antagonist during second and third instars than first instars. It is interesting to note that, none of the adults were attacked by the natural fungus. Similarly, few grubs were found attacked by endoparasitic nematodes (Mermithidae), however, majority of the larval death were largely unknown but could have been associated to unsuitable environment during captivity. This study has indicated the ample scope of studying mortality factors taking into account of other biotic as well as abiotic factors, where the most vulnerable factors could be depicted. The beetle species involved into each sites and studied in the life table are presented in Table 10.4.

Table 10.4 Composition of beetle species in life table study at Rampur, Chitwan, Nepal in 2004.

| Collection site | Morphological types | Species involvement with their scientific name |
|---|---|---|
| Gunganagar (Chitwan District) | Smaller body sizes | *Maladera affinis* (Blanchard); Melolonthinae |
| Nahala, (Tanahun District) | Medium body sizes | *Xylotrupes gideon* (L.); Dynastinae |
| Pang, (Parbat District) | Larger body sizes | *Lepidiota albistigma* (Burmeister); Melolonthinae |

### 10.4.2 Life cycle studies

Larval characteristics and their life cycle are presented in Table 10.5 and 10.6 respectively. Table 10.5 has clearly indicated that white grub larvae originated in different regions of Nepal posses different characteristics.

Figure 10.1 Study of the life cycle of white grubs in IAAS, Rampur

Figure 10.2 *Xylotrupes gideon* (Male and female, the first and second); *Maladera affinis* (third) and *Lepidiota albistigma* (fourth, the extreme right).

The larval species abundant in all the study sites are found profoundly differences in their morphology. The smaller larvae are abundant in tropical low belts of terai region in Chitwan and they are found somehow fast locomotors and aggressive compared to the larvae of low mid hills (Tanahun) and mid hills (Pang) farming. Often they want to escape

soon when put in hand and try biting within short instances. The mid hill larvae collected from Tanahun farming site were found sluggish, move somehow dorsoventrally and their body color is dull white with distinct setae along the body. Whereas the larvae collected from Parbat Districts area are larger in body size with prominent thoracic legs and faint yellow body color. This information would be relevant for the description of the adult insects.

The prominent characteristics of the larvae of the major species are presented in Table 10.5.

Table 10.5 Major characteristics of white grubs observed in the life cycle study at IAAS, Rampur, Nepal in 2004. (S = small, M = Medium, L = Large, Sl = Slow, F = Fast, D = Dorsoventral, V = Ventral, DV = Dorsoventral, Ag = Aggressive, NAg = Not aggressive)

| Location | Larvae characteristics | | | | | | | | | | White grubs species |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Body size | | | Movement | | Locomotion | | | Aggressiveness | | |
| | S | M | L | Sl | F | D | V | DV | Ag | NAg | |
| Gunganagar, Chitwan | S | - | - | - | F | - | V | - | Ag | - | *Maladera affinis* (Melolonthinae) |
| Nahala, Tanahun | - | M | - | Sl | - | - | - | DV | - | NAg | *Xylotrupes gideon* (Dynastinae) |
| Pang, Parbat | - | - | L | Sl | - | - | - | DV | - | NAg | *Lepidiota albistigma* (Melolonthinae) |

The life cycle of the common white grub species is presented in Table 10.6. Based on the result *M. affinis* were found sparsely distributed in the low hill terai area (Chitwan) of Nepal whereas, *X. gideon* in the low mid hills area (Tanahun) and *L. albistigma* in the mid hill area (Parbat) of Nepal.

Table 10.6 Life cycles of different species of white grubs from different locations of Nepal during 2003/04 at IAAS, Rampur, Chitwan, Nepal (22-23 $^{o}$C).

| Origin of the beetles and species | Life cycle duration (days) of different stages | | | | | | |
|---|---|---|---|---|---|---|---|
| | Larval duration | | | | Pupal duration | Adult duration | Total duration |
| | First instar | Second instar | Third instar | Total duration | | | |
| *Maladera affinis* (Blanchard) Tanahun | 12 b | 19 b | 27 b | 58 b | 15 b | 14 b | 87 b |
| *Xylotrupes gideon* (L.) Parbat | 13 b | 18 b | 26 b | 57 b | 12 c | 16 b | 85 b |
| *Lepidiota albistigma* (Burmeister) | 15 a | 21 a | 248 a | 284 a | 19 a | 22 a | 325 a |
| SED | 0.30 | 0.35 | 1.69 | 1.86 | 0.46 | 0.57 | 1.90 |
| LSD | 1.255 | 1.49 | 7.06 | 7.76 | 1.94 | 2.37 | 7.91 |
| CV% | 5.47 | 4.52 | 4.15 | 3.44 | 7.52 | 8.12 | 2.82 |

Figure 10.3 General life cycle of the beetle (egg, larva, pupa and adult from left to right)

The life cycle study has clearly indicated that the beetle species common in Parbat District have significantly ($p<0.001$) longer duration (325 days) compared with the insect species of Tanahun (85 days and Chitwan (87 days) (Table 10.6). Furthermore, every stage of the beetle species of Parbat District is significantly higher than the beetle species of former two Districts. It is interesting to note that the third instars of the *L. albistigma* as found in Parbat District is significantly longer (284 days) than that of the beetle species of other two study sites. The possible reason for this may be due to the involvement of the different species, crop grown, soil type, micro-climates, forest and natural habitats etc.

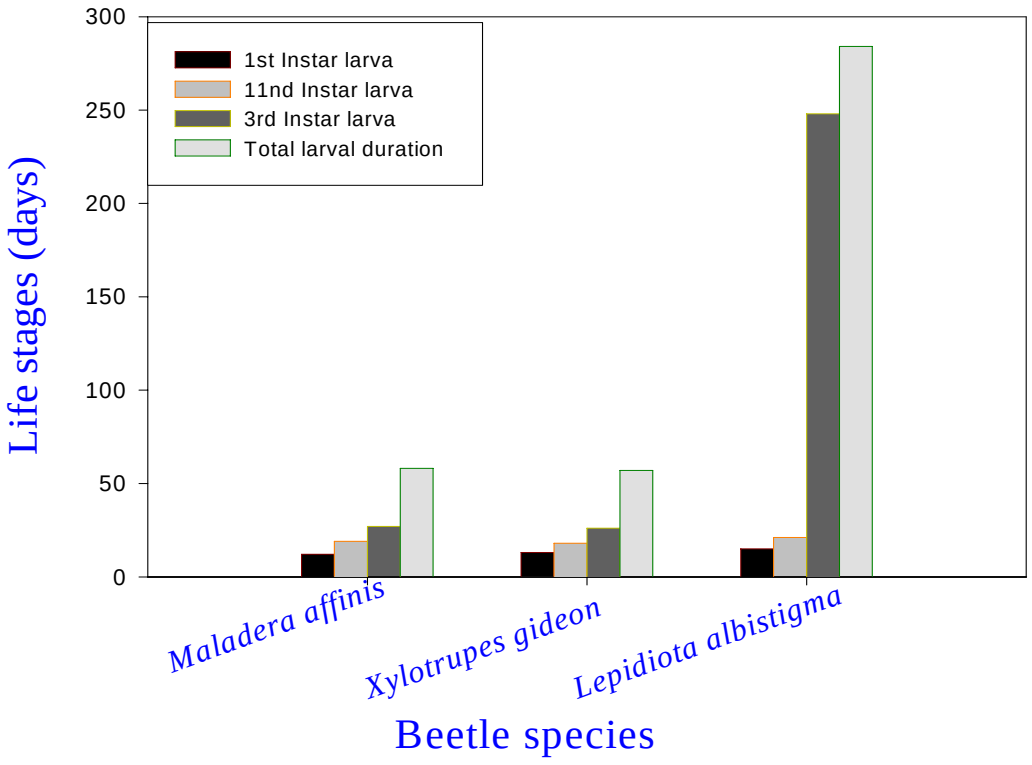



Figure 10.4 Larval duration of different species of beetle in IAAS, Rampur in 2003/04

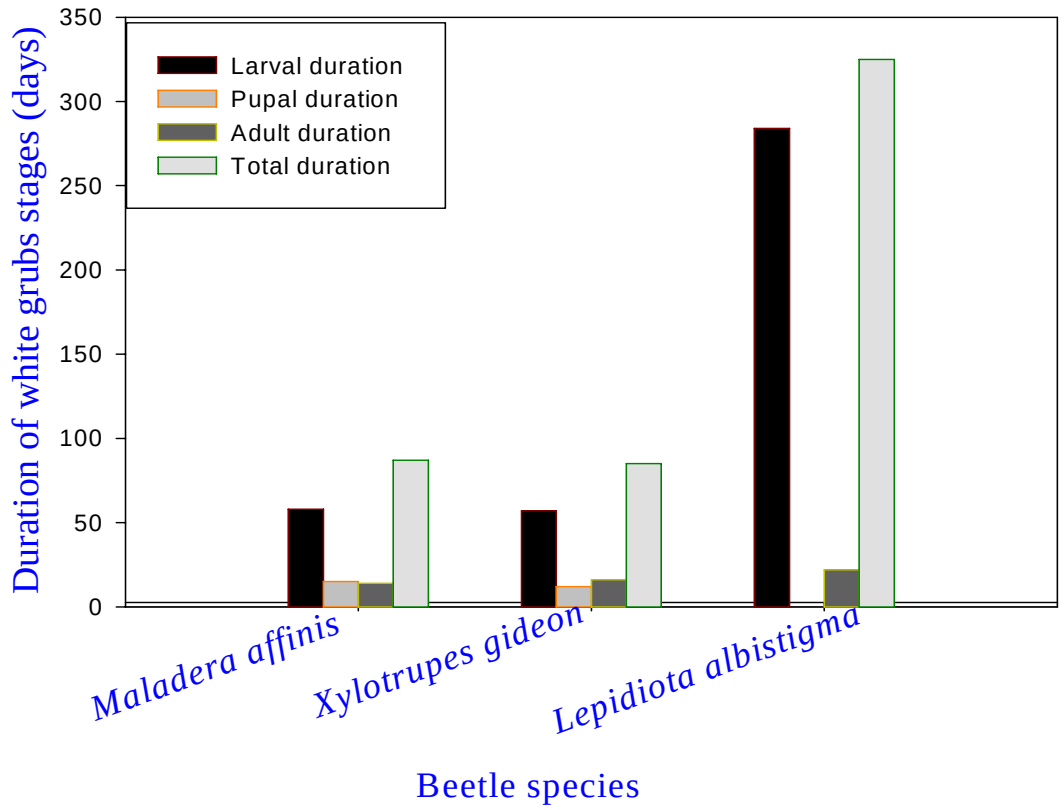



Figure 10.5 Duration of different stages of three different species of white grubs at IAAS, Rampur in 2004/05.

### 10.4.3 Activity of common beetle species in the research sites

The life cycle of the *Lepidiota* is longer than *Xylotrupes* and *Maladera*, taking more than one to two years for the insect to reach into maturity. Their life cycle in field condition could not been undertaken, however, their activity were studied through sampling in different time. The adult beetles of *Lepidiota* emerge from the soil during April and May in response to the first heavy seasonal rains, and large numbers may be collected at light at that time. *Lepidiota* includes the very large species found mostly nearby forest localities. Adults are active in the spring, usually from late May until last June. Both sexes fly in search of food, mating and such flights are also associated with oviposition. Eggs are laid in late May to early June. The newly laid egg is white, opaque, and elongate. The eggs hatch within a few weeks, and the 1st instar (or larval stage) feeds through the rest of the summer. The newly hatched larva begins to feed on organic matter and small roots. It is extremely vulnerable at this stage and even slightly unfavorable environmental conditions rapidly cause mortality. In the laboratory, mortality was highest during the first instar, when over 75% of all deaths occurred. Small grubs feed during the first summer, moving down in the soil to overwinter. In spring, the larva develops into the second instar, which moves deeper in the soil for the winter. The second instar returns to the surface in the spring and feeds throughout the summer. The second summer they feed vigorously, causing severe damage, then move down in the soil profile to overwinter the second winter. This second instar is

the life stage responsible for the most severe damage. They complete feeding the third year, overwinter as adults and emerge the following spring to complete the life cycle. Third instar larvae, which cause more damage than those in earlier instars, are present from the end of June until October. In the fall, the grubs again retreat deeper into the soil, returning to the surface in the spring as the third instar. Once fully fed they burrow down into the soil and form a cell at the first compact soil they encounter, usually at a depth of 20-30 cm. This takes place from August onwards and is completed by all individuals by November. Pupation normally takes place in January- February. The adults remain dormant in the soil until the following spring, when they emerge from late May to early June to mate.

The life activity of the white grubs' species common in the mid hill area in field condition based on the sampling in the soil can be described as below.

**Jan-Feb:** Grub in winter cell.
**Mar-Apr:** Grub comes up near surface to feed.
**May:** Grub forms cell and prepare to pupate.
**June:** Grub changes to pupa and then to adult, which emerges from ground.
**July:** Beetle lays eggs in ground, preferably in grassy area or near crop root zones.
**Aug:** Eggs hatch. Young feed on living roots of plants.
**Sep-Oct:** Grubs continue to feed and grow rapidly. Injury to roots of plants is most common at this time.
**Nov-Dec:** Grubs are mostly full grown and go to depths 8-10 inches below surface to pass winter in earthen cell.

The general activity of the *Lepidiota albistigma*, a common species in mid hill areas like Parbat District of Nepal is described with Figure 9.6.

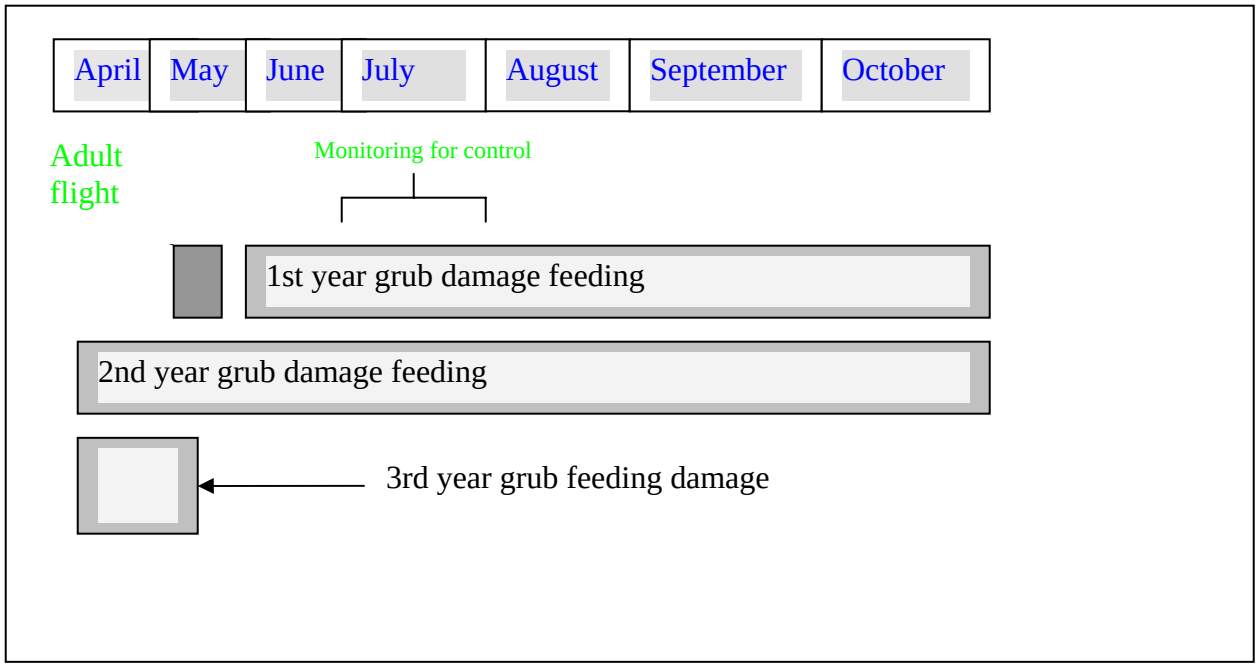



Figure 10.6 The life activity of *Lepidiota albistigma* in farmers' field in Nepal

*Xylotrupes* is very common species found in the plains as wells as mid hill area like Parbat District. They are common throughout the maize and fruit crops growing areas. These larvae are fat soft grubs, with a much wrinkled body, and as the tissues inside move. The larvae behave like typical cockchafers grubs, feeding on the root of the plants. Adult male often are horned whereas female are hornless. Unlike, former species these beetle complete their life cycle in a year, however, their damage in field condition found mainly during rainy season and occur every year. Based on field sampling, their activity is generalized with the Figure 10.7

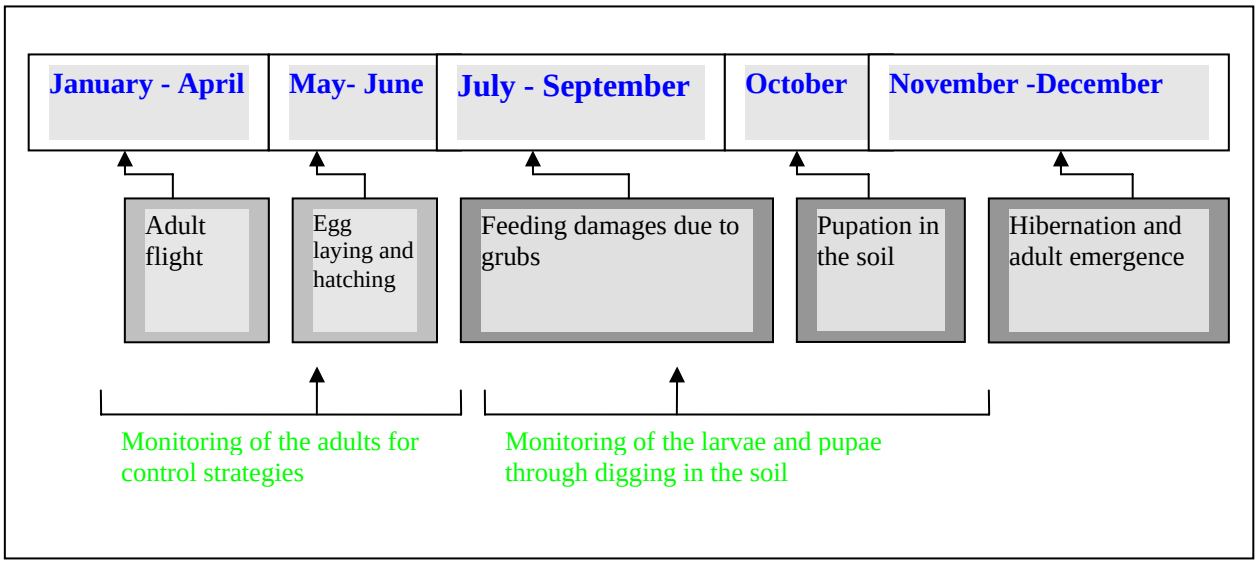



Figure 10.7 Timing of *Xylotrupes gideon* life activity in field condition in Nepal

The *Maladera* are smaller beetles commonly found in dry, sandy soils of mostly terai condition of Nepal. The larvae have well developed segment in the head capsule which allows them to recognise easily. In general they are found singly and infrequently. They appear annually and complete their life cycle in one year. Adults are tan brown, about 5-8 inches long, and slightly smaller than the three-year white grub adult. Adults are normally present from late June to July. They are attracted to lights and are frequently observed around windows or porch lights. Adults deposit eggs in the top inch or two of soil, often in small clusters. Small grubs hatch from the eggs and being feeding on grass roots. Most damage occurs in late summer and early fall after the grubs have reached the second larval stage. With the onset of cold weather, grubs move deeper in the soil to overwinter. As the soil temperatures warm in the spring they move up to the grass roots, feed for a short time, pupate, and emerge as adults to begin a new cycle. They are common in the low hill terai region of the Chitwan and Nawalparasi condition:

In the normal life cycle of this species, the adult females lay eggs in late June to early July. The eggs hatch in late July and larvae mature by October, when they move down into the soil to overwinter. In March or April, the grubs return to the surface and resume feeding. In early May, the grubs pupate and develop into the adult beetle, which emerges in late June to mate. In general, the timing of beetle activity can be summarised as follows:

**Jan-Feb:** Some species of the grub in winter cell and some grub come up near surface to feed.
**Mar-Apr:** Grub comes up near surface to feed and some lay eggs.
**May:** Grub forms cell and prepare to pupate whereas, other larval species feed continue
**June:** Grub changes to pupa and then to adult, which emerges from ground and start laying egg.
**July:** Beetle lays eggs in ground, preferably in grassy area or near crop root zones.
**Aug:** Eggs hatch. Young feed on living roots of plants.
**Sep-Oct:** Grubs continue to feed and grow rapidly. Injury to roots of plants is most common at this time, some start pupating.
**Nov-Dec:** Grubs are mostly full grown and go to depths 8-10 inches below surface to pass winter in earthen cell. The general life activity of the *Maladera* in field condition is summarized in the Figure 10.8.

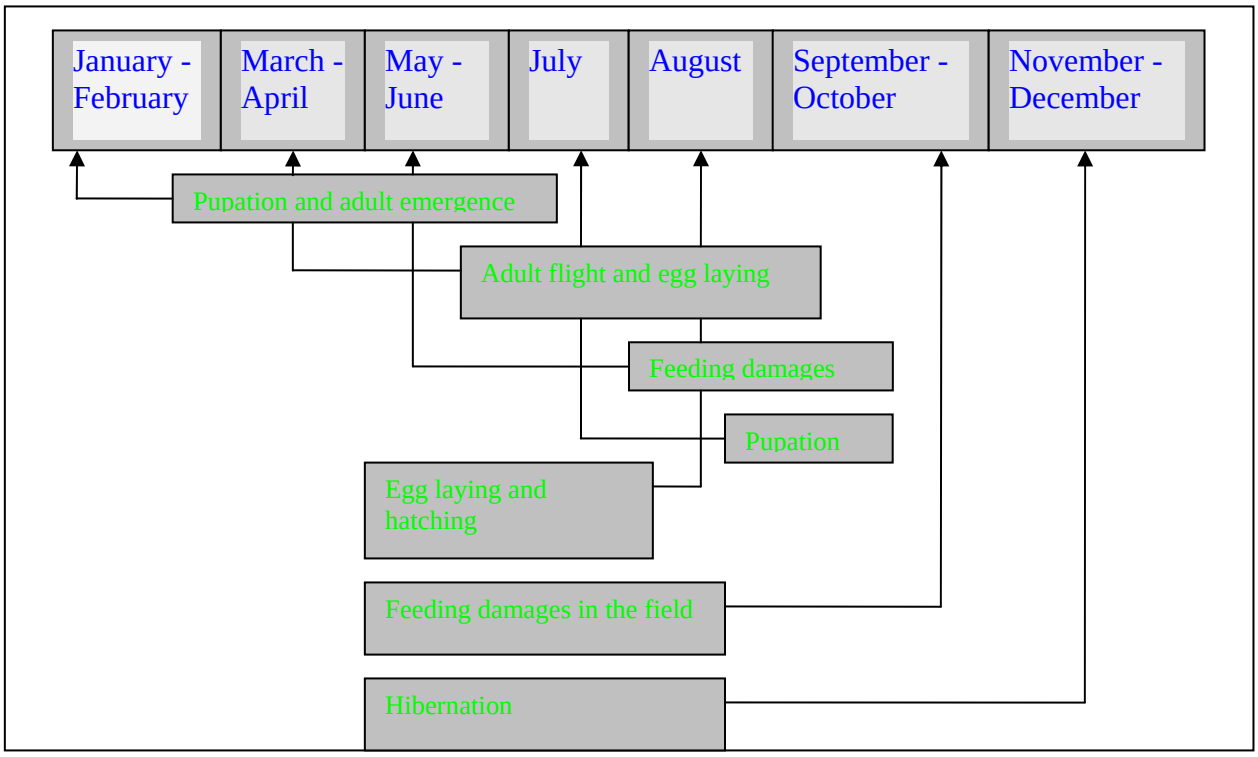



Figure 10.8 Timing of *Maladera affinis* life activity in farmers' field

## 10.5 DICUSSION

Considerable numbers of mortality factors are associated in the natural environment which is detrimental to all stages of the insect; however, early instars larvae are highly vulnerable. The chances of disturbances in the life process of beetles are particularly higher during immature stages than that of dormant and mature stages. Higher mobility and vulnerable morphology of the larvae could be some of the attributing factors, whereas, the matured insect are equipped with defense mechanisms and somehow resistance with the antagonists and adverse environmental conditions. In this study, small fractions of white grub's larvae are also being attacked by natural funguses as well as endoparasitic nematodes, however,

there could be immense number of other antagonists, whose role could not been recognized. Particularly, the role of bacterial infection was difficult to assess because of the rapid loss of the decayed cadavers.

Insect species common in high mid hills area take longer duration for completing the life cycle than the beetle species found in the lower hills of Nepal. The reasons behind this could be due to involvement of different species in different agro-environment, presence of natural vegetations, climates, soil factors, other environmental factors etc. The beetle species having shorter life cycle may cause frequent and regular damages because of their overlapping generations in contrast to the larvae having longer life cycle. The occurrence and associated damages in particular crops and areas may be attributed due the difference in life cycle and species involvement.

The timing of application of microbial pesticide is critical if control is to be effective. The best time to apply microbial pesticide is when the grubs are actively feeding near the soil surface. Therefore, study of beetle and larvae activity in the field condition is very important. As indicated in life cycle table, the feeding occurs in April through early May and from August through October especially in low hill area. The life cycle is considerably shorter with the grub species involved in these areas compared with the species of mid and high hills. Two peaks are appeared with former species whereas only one in the later species in the same site. Microbial pesticide when applied to the soil, infects the grubs and produces a disease therefore, if they applied any other time will be ineffective.

## 10.6 CONCLUSIONS

The findings of life table studies can be summarized that if the natural mortality factors would not been involved, the total life span of the beetle species and their population would be high enough because of the several overlapping generations. Similarly their damages may be considerably higher with the involvement of larger number of beetle species. Understanding of pest dynamics in relation to natural mortality factors is very important for the successful management of insect pests. The role of such regulating factors should be properly considered while initiating any biological control programmes. In addition to the presence of natural antagonists, there is necessity of deliberate intervention either through augmentation or inundation to suppress the large number of insect population.

The life cycle study has demonstrated that these species may take more than one year for completing life cycle and damage may happen in cyclic basis and not every year. Whereas, in another hand, the insect species of low tropical areas can produce more than one generation a year and they occur more or less in two seasons of a year. Based on this evidence it can be concluded that the insect species having shorter generation may induce higher crop losses compared to the insect species that has longer life cycle. Management strategies therefore, should be coincided according to their life cycle. Furthermore, intensive management is necessary to induce the natural epizootic where the insect pests complete their life cycle reasonably in shorter period of time. This study has indicated ample opportunity for selective application of the control methods based on the life stages and flight period of the insects.

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# Chapter 11

## Population dynamics of white grubs in maize based farming systems in some agro-ecological zones in Nepal

# Chapter 11

## Population dynamics of white grubs in maize based farming systems in some agro-ecological zones in Nepal

### 11.1 SUMMARY

Field studies in Gunganagar (Chitwan) provided estimates of annual losses of Scarabaeid larvae from 12-35% however, in epidemics this figure raises depending on the season and locality. Farmers may underestimate the role of these soil pests as only (16%) surveyed in Gaindakot (Nawalparasi) site mentioned white grubs as a pest (Field survey Gaindakot, 2004), whereas, 90% did mention this as major pest in Chitwan. Collection of beetles from field samplings since 2003-2005 revealed the large numbers of beetles are available in Nepal. The study so far indicated more than two dozens beetles species in the sampling sites with major being *Maladera affinis* Balanchard, *Adoretus lasiopygus* Burmeister, *Anomala dimidiata* Hope, *Heteronychus lioderes* Redtenbacher, *Anomala bilobata* Arrow, *Anomala xanthoptera* Blanchard, *Maladera cardoni* Brenske, *Idionychus excisa* Arrow, *Anomala cantori* Hope, *Mimela silguria* Arrow. These species occur lesser or greater extent annually in both the study sites.

In 2004 and 2005, large numbers of adults were seen clustered from April-July in fodder trees which are protected by farmers in their farmland. Of the common species the life cycle was studied only with *M. affinis* where there is no evidence of dormancy in any stage (Chapter 9). Flight trees provide food as well as serve as an aggregation site for mating. Looking into the flight activity of beetles during sampling, it was decided to initiate a study on the preferences for fodder trees by different beetle species with a hope of obtaining a ecological basis for future control measures. More than a half dozen of fodder trees are found preferred by beetle species, however, the dabdabe (*Garuga pinnata* Roxb*)* is more preferable over other fodder species.

The population dynamics through digging indicates, soil depth up to 20 cm is most preferable depth for larval activity, whereas eggs are mostly laid up to 15 cm. The pupae and adults is concentrated somehow deeper layer and mostly found during winter months. With this study two major peaks such as March-April and June-July were found for the larval activity in terai conditions. The possible reasons may be due to overlapping generations and involvement of many species in the same environment. Similarly, the pupae and adults observed during winter months and only very few species such as *An. dimidiata* and few other congregate in tree plants for breeding purposes and majority of them held breeding unnoticingly. Control options, therefore may be adopted either to the beetle (however, allows very few possibilities) or larva based on their abundance and availability.

### 11.2 INTRODUCTION

In Nepal, white grubs, the larvae of Scarabaeid beetles are regarded as major production constraints in upland farming. In recent year their infestation has been widespread across the country. In this sense, white grubs are regarded as one of the nationally important pest for maize based farming (Pokhrel, 2004) that occurs year after year in some locations

whereas in erratic trends in other locations (NMRP, 1997). Among many unknown reasons for their severity, the impact of the improved farming has been blamed by majority of the farming communities. In farmer's perception, these practices have led into the multiple cropping and increasing tendency of soil fertilization with the addition of many foreign products. Elsewhere, white grub's problem has believed to be favored with the heavily sloping dry and nutrient depleted acid soils coupled with the addition of un-decomposed farm yard manure (FYM). In order to provide scientific bases on these hypotheses, a study on the white grub's dynamics has been initiated in the central region of Chitwan and Nawalparasi Districts of Nepal.

Association of adults to the plant species (mainly *An. dimidiata* and *Ad. lasiopygus*) were recorded during the observation. Occurrence of the adult of *A. dimidiata* is the best known among the common white grub species however; digging experiment during two years 2003-2005 revealed that *Maladera* sp and *Ad. lasiopygus* concentrate abundantly in root portion of maize crops. In the same way, *An. dimidiata* including other species of *Anomala* are observed across the study sites, however, *Maladera* were found mainly concentrating in the low lands (terai) of Nepal. Nevertheless, the larvae of these beetles involve in different plants as well as weed species. White grubs in the genera *Anomala* and *Adoretus* are members of the subfamily Rutelinae, while *Maladera* and *Heteronychus* are the members of Melolonthinae and Dynastinae respectively. Both subfamilies share similar habits in that all members are phytophagous and possesses three larval stages that feed on humus or roots mainly of grasses while adults feed on leaves or nothing at all (Ritcher, 1958). Some Rutelinae larvae prefer humus to live roots but will feed on the later when humus is lacking (Wilson, 1969). Among the root-feeding species, the first larval instar (L1) stage survives better on humus than a diet of live roots, while live roots are needed by the L3 to puapate (Wilson, 1969; Veeresh, 1977). Due to its size and longevity most damage is done by the L3 (King, 1984).

Until recent past effective management for white grubs attack is lacking in Nepal because of the poorly understood bioecology in the soil environment and alternatives to chemical compounds. Furthermore, understanding of pest biology is very much important for the successful planning of the pest population. In Nepal, such studies are lacking and most of the controlled operations are mainly on the guesstimate resulting into the achievements of very poor successes of pest control. Looking into this lacuna, systematic works on the understanding of white grub's dynamics were initiated with field sampling by digging. The study was carried out for two years in two different farming areas with effect from 2003 through 2005. In the same area, assessment of insect pathogenic fungi was also carried out as a means to white grubs control in the first time in the country.

The observation parameters in this study include the white grub's density at different soil depths, species composition, understanding of larval phenology and adult emergences etc. The studies were focused on population dynamics of larvae, pupae, and adults from soil samples which will be used as a basis for timing control measures. Depth of grubs in the soil was measured to learn the potential for tillage as a cultural control. Adult preferences for tree species were investigated to provide information on the feasibility of adult control. Laval abundance was measured among the major cultivated plots and uncultivated meadow to evaluate the potential of fallow land management. The major objective of the study was to observe the dynamics of the common damaging white grub species in different production

system so as to make possible to forecast of the infestation and determine the appropriate application time of the fungus in the field. In general, the information on population dynamics would make possible to take more appropriate measures to protect the crop.

## 11.3 MATERIAL AND METHODS

### 11.3.1 Description of the study sites

The study sites are located along the catchments of Narayani River from an elevation of 230 m asl. (Gunganagar) and 205 m asl (Gaindakot). These areas are presented in chapter 8 in the Figure 8.1. The area in the former site was deforested several generations previously and immigrants, mostly from the newly settlers during 1950s. The forest has been replaced to crop lands richly surrounded by grasses (*Imperata cylindrica)* with scattered dominantly with sisso (*Dalbergia sisso)* and several species of tropical fodder trees. The area was dominated by cattle ranches but is slowly being converted to small holdings by immigrant mainly planting maize and vegetable growing are also increasing for the urban markets. There is more cultivated than uncultivated land in former site whereas vice versa in later site. Rainfall averages 1105.3 mm/year with the amount increasing with elevation. The normal rainy season, based on the records of NMRP (2004), normally begins from April- May averaging 180.0-111.4 mm/month followed by the highest rains (averaging 214.3-417.7 mm/month) from August-September. The dry season with no rain occurred in December, February and March, however very few i. e. 62.7 mm/month in January. The monthly average temperature, rainfall, relative humidity and soil temperature during 2003-2005 was measured daily to the nearest mm from the nearby meteorological recording of National Maize Research Program (NMRP). It was averaged to the monthly basis and is presented in Appendix 11.1. In addition to maize as a sole crop, the community people intercrop several legumes followed by sesame (*Sesamum indicum*), minor vegetables, sugarcane (*Saccharum officinarum),* zinger (*Zingiber officinale*), turmeric (*Curcuma longa)* etc. Ornamental plants, tropical fruit mainly banana (*Musa paradisiaca*), pineapple *(Ananus comosus)* and other trees are scattered throughout the area.

### 11.3.2 Study on the natural vegetation for beetle preference

During heavy emergence of beetles, their associations into preferred plants were recorded from among the common trees available in the area to determine adult host preferences. Adults were seen mating and resting in the tree during the recording of 2004. As a result, it was decided to sample the adult population that prefers to come in above ground portion of plants during main season of 2005. Thus, the major plant species common in Gunganagar and Gaindakot research sites were monitored twice a week from April-August 2005. During this period a total of 10 samplings were made into two different sites. On each sampling date, plastic sheets were spread below each tree and the branches shaken one by one to dislodge the beetles. During hunting, some people positioned themselves around each tree to record the number of beetles that flew away while other assistance collected the beetles that dropped onto the sheeting. Along with the information on natural vegetation in the study site, environmental parameters were also recorded.

### 11.3.3 Site characteristics and soil depths

The experiment was carried out in two different localities with different site characteristics. One of the study site include the heavily cropped area within Gunganagar Village Development Committee (VDC) of Chitwan District whereas, another site was located in the river basin forest area within Gaindakot VDC of Nawalparasi District. The sampling depths were measured with a meter wooden scale to the nearest cm across the pit level with the ground surface. In order to understand the white grubs density, species prevalence with regard to different soil depths and season, digging was carried out at six different depths which includes, 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70 cm with a diameter of 1 $m^2$ area. The digging was continued until two years at biweekly intervals and all the collected insect's stages were reared at 22 $^oC$ in the insect pathology laboratory of IAAS, Rampur for further investigation.

Table 11.1 Description of the habitats for biweekly sampling of white grubs over two years period (November 2003-December 2005) in Nepal.

| Habitat | Cultivation | Exposure | Topography | Area (ha) | District |
|---|---|---|---|---|---|
| 1 | Round year cultivation | Semi open | Flat, North facing | <0.5 | Chitwan |
| 2 | Round year cultivation | -do- | -do- | -do- | -do- |
| 3 | Round year cultivation | -do- | -do- | -do- | -do- |
| 4 | Round year cultivation | -do- | -do- | -do- | -do- |
| 5 | Partly cultivation | Open | River basin, East facing | <0.5 | Nawalparasi |
| 6 | Partly cultivation | -do- | -do- | -do- | -do- |
| 7 | Partly cultivation | -do- | -do- | -do- | -do- |
| 8 | Partly cultivation | -do- | -do- | -do- | -do- |

### 11.3.4 Population density in relation to soil depths during 2003/04

Sampling with digging of the soil was carried out at biweekly interval until two years i.e. starting from November 2003 through November 2005. Soil sampling was stratified from eight different replications in two villages (Gunganagar and Graindakot) for a total of 16 soils pit per digging per date in one site. This means a total of four plots were dug on the basis of four holes per plot per day as per the specified depths. A sample consisted of one square meter area of soil carefully dug by shovel and searched by hand for eggs, larvae, pupae, and adult stages. Eggs were too small thus they were tedious to be sampled; however, they were recorded so far as possible. Sampling sites were selected within each permanent site by randomly throwing a 1 m x 1m quadrant by hand. All the life stages of beetles recovered during samplings were placed in plastic jars (4.5 cm diameter and 6 cm height). The lids were perforated for aeration and soil of the same site was added to allow their growth. They were taken into the rearing room at the insect pathology laboratory of IAAS, Rampur and made adults upon feeding the slices of potato for further identification with the aid of a dissecting microscope.

During samplings, all the stages of white grubs collected from various depths were immediately marked with their exact origin based on their depths, date, replication etc. Eggs were allowed to hatch into larvae with very poor successes; however, larvae were reared into further stages with modest successes. The adults recovered during digging as well as obtained from rearing the larvae were identified later with the help of identification keys and reference insects.

### 11.3.5 Population density in relation to soil depths during 2004/05

Similar to previous year, different stages of white grubs were recorded during 2004/05 in the same sites however, at different spots. In order to know the population dynamics, biweekly sampling was carried out by digging with a shovel. The total grubs were calculated with the cumulative numbers of the individual sampling. Different stages of beetles from different depth was totaled which are presented in graphical as well as tabular form in the following sections.

### 11.3.6 Species prevalence in relation to soil depths during 2003/04

Because of the lack of identification keys for larvae, all the stages were reared into the laboratory until their adult emergence. After rearing them as adults, they were preserved into 98% absolute alcohol and identified later with the identification keys provided by the expert Prof. Dr Peter Nagel, major advisor and Dr Dirk Ahrens, consultant entomologist. The identified beetle species are preserved into reference collection at the Insect Pathology Unit of Entomology Department of Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan, Nepal.

### 11.3.7 Species prevalence in relation to soil depths during 2004/05

In order to know the species prevalence, similar method as followed in the previous season was adopted in this year. The collected beetle stages were made adults and they were identified later on. The major species involved at different soil depths over the period of 2003-2005 are presented in the following sections.

## 11.4 RESULTS

### 11.4.1 Natural vegetation for beetle preference

Considerable numbers of beetle species are abundant in the study area, however, a handful of them harbor some of the plants either for mating or food or shelter etc. The major plant species preferred by beetle species and their association with cumulative number over the period is presented in Table 11.2 and Appendices 11.2-11.4.

Table 11.2 Most prevalent plant species preferred by adult beetles and their association* over the recording period in Gunganagar and Gaindakot, Nepal, 2005. * Cumulative number of the beetles of ten sampling dates and two replications per tree species /sampling unit area; ** In case of maize and millet a total of 4 m x 4m area was fixed for sampling

| SN | Plant species | | Occurrence of beetle species by months (cumulative number from 10 samplings) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Local Name | Scientific Name | *An. dimidiata* | Total | *H. nigricollis* | Total | *H. liioderes* | Total |
| 1 | Dabdabe | *Garuga pinnata* Roxb. | Apr- Aug | 313 | Apr- July | 198 | Apr- Aug | 0 |
| 2 | Neem | *Azadirachta indica* L. | Apr- Aug | 228 | May-July | 101 | Apr- Aug | 0 |
| 3 | Acalifa | *Acalypha* spp L. | May-Aug | 221 | Apr- May | 45 | Apr- Aug | 0 |
| 4 | Rose | *Rosa alba* L. | Apr- Aug | 201 | May-July | 41 | Apr- Aug | 0 |
| 5 | Baharamase | *Calotnopia gigantea* L. | Apr- Aug | 193 | Apr- Aug | 40 | Apr- Aug | 0 |
| 6 | Maize** | *Zea mays* L. | Apr –June | 191 | Apr- July | 36 | Apr- July | 144 |
| 7 | Litchi | *Litchi chinensis* Sonner. | May-July | 54 | May-Aug | 49 | Apr- Aug | 0 |
| 8 | Mulberry | *Morus indica* L. | July-Aug | 43 | July-Aug | 36 | July - Aug | 50 |
| 9 | Khanyu | *Ficus cunia* Buch-Mam | May-Aug | 39 | May-Aug | 78 | Apr- Aug | 0 |
| 10 | Bakaino | *Melia azadarach* L. | May -July | 24 | May-Aug | 40 | Apr- Aug | 0 |

Following are major vegetation in farmer's fields, which are mostly preferred by *A. dimidiata* and the beetle congregates on them either for food or shelter or copulation.

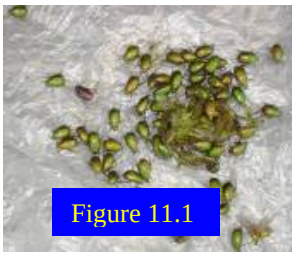


Figure 11.1

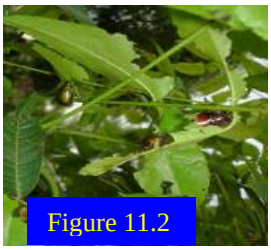


Figure 11.2


Figure 11.3


Figure 11.4


Figure 11.5

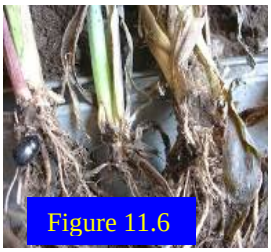


Figure 11.6


Figure 11.7


Figure 11.8

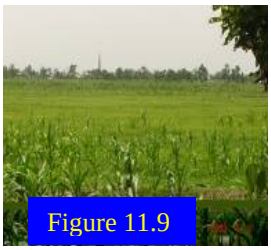


Figure 11.9


Figure 11.10

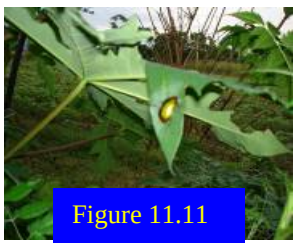


Figure 11.11

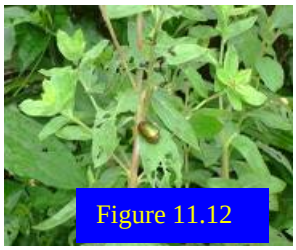


Figure 11.12

Figures 11.1-11.12 Seasonal occurrence of adult beetles in different vegetations in Gunganar and Gaindakot, study sites during 2003-2005 (11.1: Swarm of *A. dimidiata;* 11.2 Damage on *Garuga*

*pinnata*; 11.3: Damage on *Acalifa;*11.4: Damage on rose; 11.5: Damage on cabbage plot; 11.6: Devastating problem of *H. liioderes* on maize plots; 11.7: Damage on flower; 11.8: Damage on Baharmase; 11.9: Damage on maize plot due to *H. liioderes*; 11.10: Damage on okra; 11.11 Damage on *Carica papaya;* 11.12 Makhamali flower)

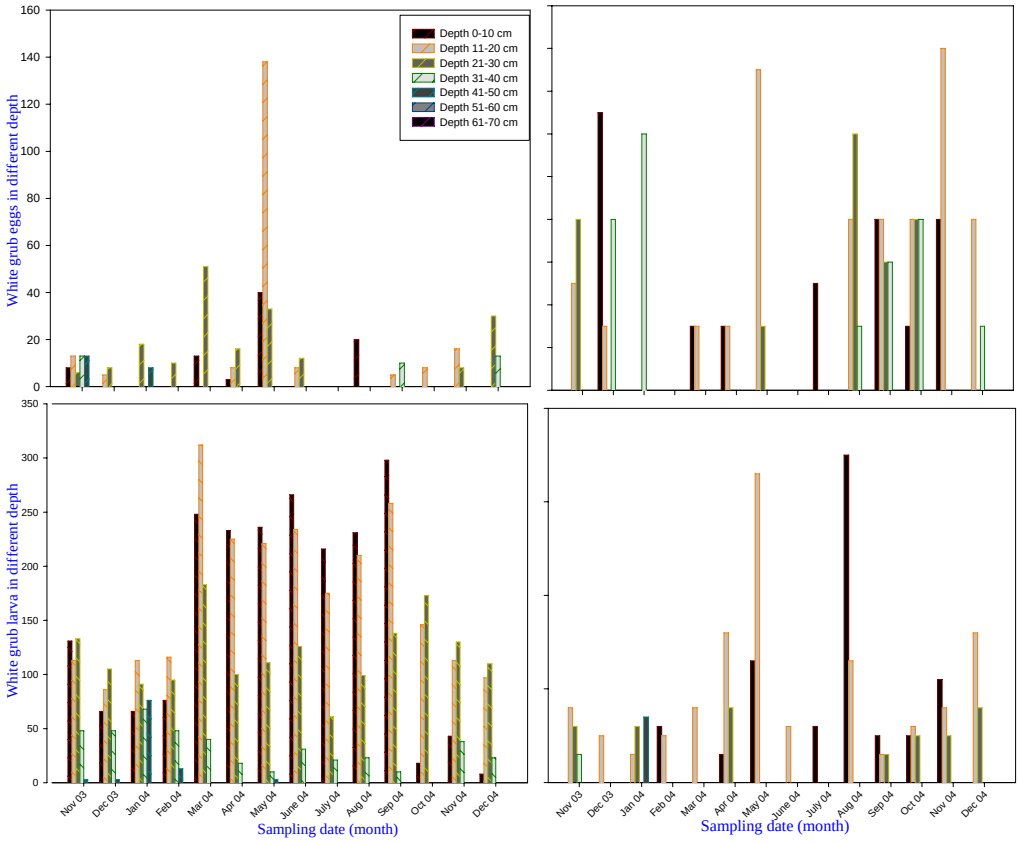
Among them, large number of *Anomala dimidiata* was noted of involving in dabdabe (*Garuga pinnata* Roxb.) as one of the preferred host plants followed by other species and the least to mango (*Mangifera indica* L.). Similarly, *H. nigricollis* also congregate more on the similar types of plant species however, with less frequency. Interestingly, *H. liioderes* confine mainly to the crop plants during seedling stages and none of them were recorded in other plant species. During digging in August-September these species were found residing inside the soil mainly in adult stages. In addition to these plant species number of other plants were found harbored (Figures 11.1-11.12) by *A. dimidiata,* however, with very less frequent occasions, these includes, okra (*Abelmoschus esculentus* L.*)*, papaya (*Curica papaya* L.), jackfruit (*Artocarpus heterophyllus* Lam.), belhar (*Trewia nudiflora* L.), sisoo (*Dalbergia sisoo* Roxb.), peach (*Prunus persica* L.), guava (*Psidium guajava* L.), rudrachya (*Elaeocarpu shaericus* Gaertn.), bamboo (*Dendrocalamus* spp. Nees and Arn.), chilaui (*Sachima wallichii* Korth), ficus (*Ficus sarmentosa* Buch.), kutmiro (*Listea monpertala* Roxb.), kharseto (*F. hispida* L.) etc.

Their seasonal occurrence was also confirmed by local residents and sampling at biweekly intervals. Because of the differences in size, color and behavior of the three species which could be identified either as an *An. dimidiata* or a *Holotrichia nigricollis* or a *Heteronychus liioderes* or other species. The former species is faint green in color, has moderate body size and short fliers, whereas, *Holotrichia* are brown in color with smaller body size and rapid fliers. In addition to these two species, *Heteronychus* involves massively in maize seedlings before crop matures. Their damages are noticeable in the seedling stages of the maize around the soil surface. Among the beetle species abundant in plant species in the study area, *An. dimidiata* was found involving frequently in different plant species. Next to this, *H. nigricollis* also harbors more or less in similar plants however with some occasion by far more comparatively lesser extent. *Ad. lasiopygus* was found to be involved mainly in crop plants. In general, beetles were found more actively involved in crop plants during April through August over the recording period into in different vegetation.

### 11.4.2 Population density of white grubs in relation to soil depths during 2003/04

The biweekly sampling was averaged to monthly population to better note trends in population dynamics of the egg, larval, pupal, and adult stages over the entire recording period. During this period all the life stages (egg, larva, pupa and adults) of the beetles recovered in relation to different season of the year and soil depths in Gunganagar research site is presented in Figures 11.13-11.16.

The number of different stages of beetles (egg, larvae, pupae and adults) of both of the research site is presented in the Appendices (11.5-11.8). Only during 2003/04 samplings, almost 8000 different stages of the beetles were excavated. Larvae were by far the highest (6734) followed by eggs (534).

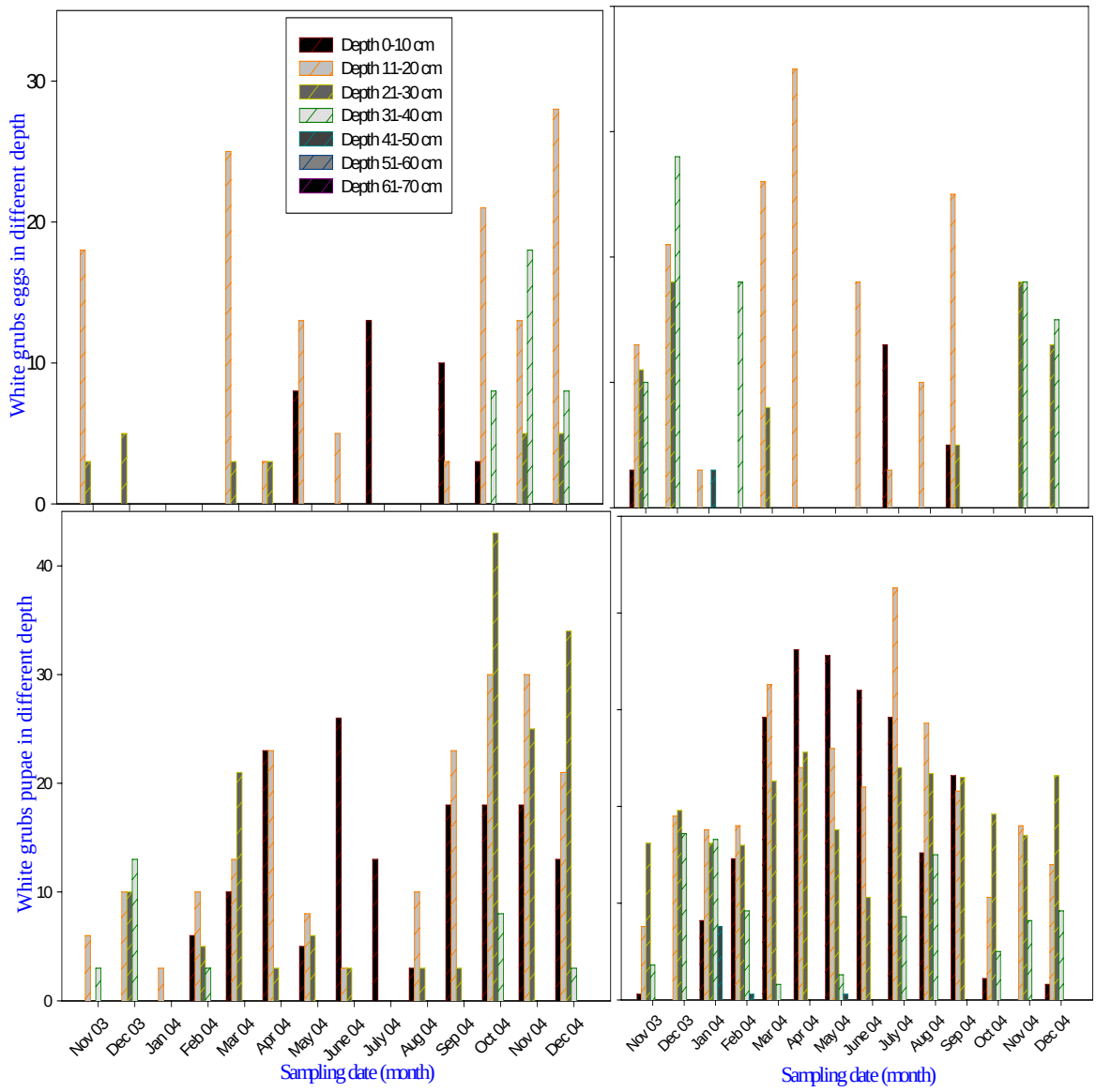



Figures 11.13-11.16 Number of eggs, larvae, (vertically left) pupae and adults (vertically right) in different months in Gunganagar, Chitwan during 2003/04.

The occurrence of the number of adult beetles (264) and pupae (197) were more or less same during November 2003 through December 2004. Larval stages with regard to different instars were not separated since the L1 stages were often overlooked due to their small size, however; L2 and L3 are larger in size and readily detected in the soil.

Larvae therefore, were collectively recorded irrespective of their instars. These stages occupy the large proportion representing the highest occurrence (84.17%) followed by eggs (6.67%) while the adults and pupae represented 3.3% and 2.46% of the total population respectively, due to their short duration in the soil. Abundance of the different stages of beetles at various soil layers, clearly showed that the highest density of eggs were recovered from the depth of 10-20 cm soil layer in May (14/ $m^2$ area) followed by March (5/$m^2$ area) whereas June and July are observed as lean period (no eggs in July) in terms of occurrence of the eggs in Gunganagar research site.

The abundance of the larval density in Guganagar showed that, March and September are the two peaks of a year where the highest numbers of larvae (32 and 30/ $m^2$ areas respectively) were found.




Figures 11.17-11.20 Number of eggs, larvae (upper row), pupae and adults (lower row) in different months in Gaindakot, Nawalparasi during 2003/04.

Whereas the occurrence of larval density in Gaindakot research site followed slightly different pattern with 18/ $m^2$ area in April and 21/ $m^2$ in July. Interestingly, these stages were found in the similar depths in both the sites. The result has further clarified that the white grub density is low during winter season compared to summer. This has also been evidenced by the fact that the larval abundance is higher by threefold during summer months compared to winter.

In the same way, the abundance of larva in relation to soil depth has clearly followed the similar trends with that of eggs where none of the larval species are available beyond 50 cm deeper layer. It is noteworthy that some larvae hibernate up to deeper layer of 30-40 cm because of the low soil moisture in upper layer during winter period.

This could have been influenced by the low or no availability of the host crops mainly during these periods. In general, crop root zones are found the preferable soil layer for white grub's abundance. Compared to the Gunganagar research site, another forest and river basin research site, Gaindakot has excessively lower number of eggs i.e. $4/m^2$ area (Figures 11.17-11.20). The availability of the eggs in relation to soil depths has clearly indicated that the highest numbers of eggs were recovered from upper layer ranging from 10-20 cm, however; none of the beetle species lay their egg beyond 50 cm depths (Table 11.3).

Interestingly, some unknown species of beetle have found the eggs laid up to 30-50 cm depths; however, such eggs were recorded only in few occasions of winter months such as November and December. The availability of pupae and adults of the white grubs showed the similar trend of abundance with those of previous generations in both the research sites. Both of the stages were abundantly common in upper layer and none of them were found below than 50 cm depths. It is interesting to note these stages are available nearly round the year. This evidence may largely support the involvement of the complex species of white grubs in the same locality throughout the year. This situation might have further aggravated by the fact of mono-cropping, traditional farming with the incorporation un-decompose farm yard manure, host plant abundance, possible pest resurgence etc. The detailed of the different stages of beetle species in Gunganagar and Gaindakot research sites during 2003/04 is presented in Appendices 11.5 and 11.6 respectively.

Table 11.3 Number of different stages of the beetles with respect to different depths in Gunganagar, Chitwan and Gaindakot, Nawalparasi, Nepal during 2004. (G/Nagar = Gunganagar and G/Kot = Gaindakot written as short form)

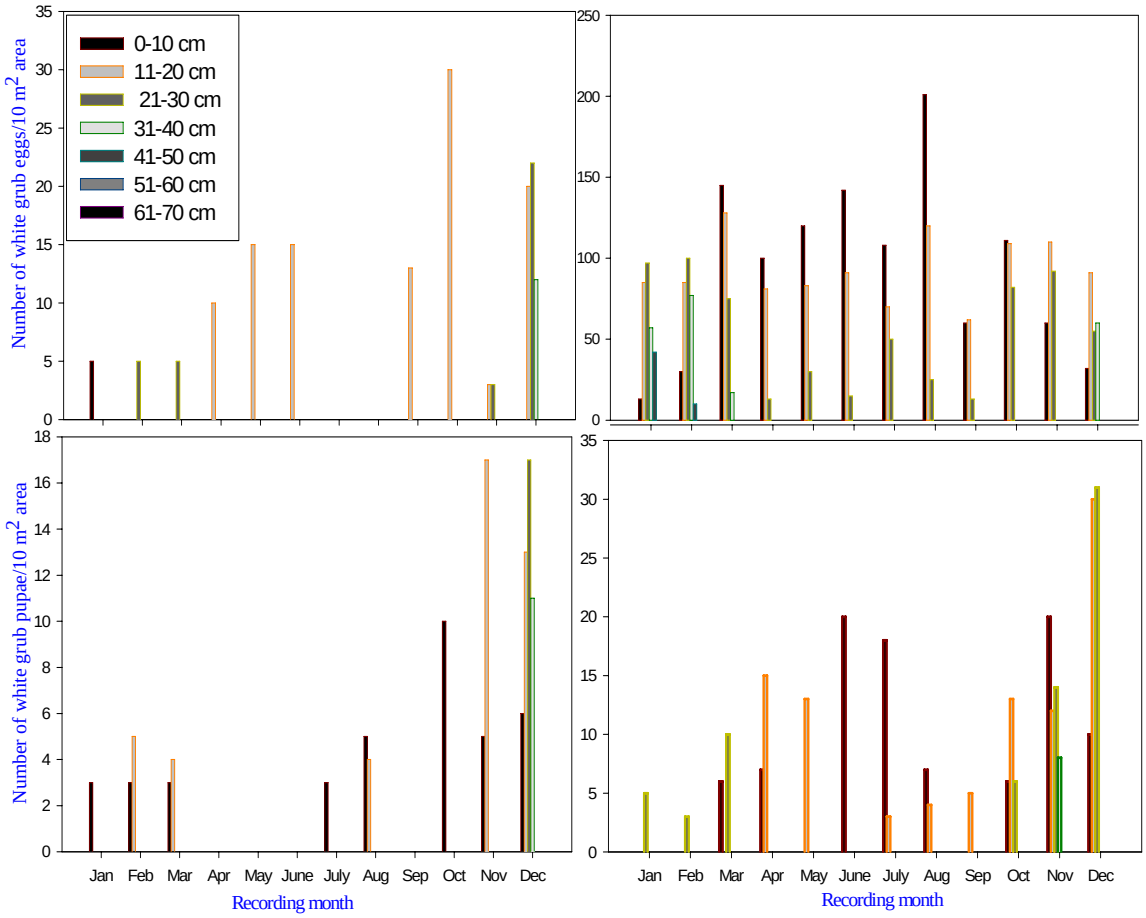
| Depth (cm) | Number of different stages of white grubs with respect to different depths (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | | Larvae | | Pupae | | Adults | |
| | G/ Nagar | G/ Kot | G/Nagar | G/kot | G/Nagar | G/ Kot | G/ Nagar | G/ Kot |
| 0-10 | 3 | 1 | 71 | 38 | 1 | 1 | 2 | 5 |
| 10-20 | 7 | 5 | 83 | 50 | 2 | 4 | 4 | 7 |
| 20-30 | 6 | 2 | 55 | 45 | 1 | 1 | 1 | 5 |
| 30-40 | 1 | 3 | 15 | 16 | 1 | 1 | 0 | 1 |
| 40-50 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 0 |
| 50-60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60-70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD | 2.85 | 1.684 | 6.65 | 4.000 | 1.17 | 0.919 | 1.12 | 1.423 |
| SEM | 1.02 | 0.606 | 2.39 | 1.440 | 0.42 | 0.331 | 0.40 | 0.512 |
| CV% | 463.3 | 422.7 | 81 | 73.3 | 546.1 | 358.2 | 404.6 | 226.5 |

### 11.4.3 Population density of white grubs in relation to soil depths during 2004/05

Depending on the white grubs density, indicators of generations can best be seen from soil sampling of pupae and adults as well as appearance of L1 and L2 stages. A number of white grub species are known to pass unfavorable dry seasons or winters as inactive (but not diapausing) pre-pupae, pupae, and eventually adults in pupal cells with undeveloped reproductive systems and large fat bodies. In this study, none of the grub stages were found as dormant probably because of annual life cycle. Similar observation was found in the laboratory rearing where the extended larval period was found probably due to unfavorable

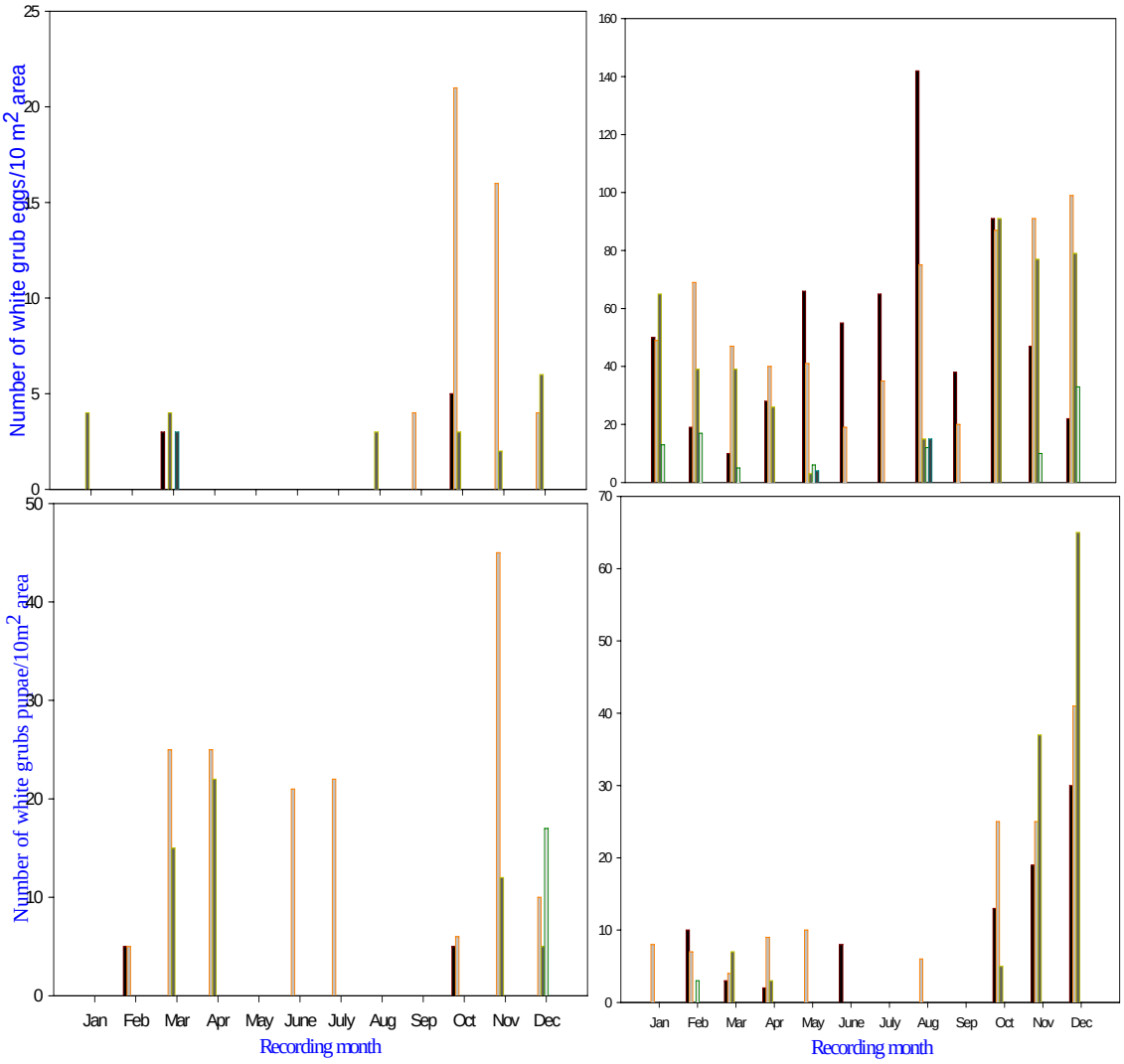
environment in the field. When activity resumes, the reproductive systems mature and adults emerge from the soil in response to rainfall to aggregate and mate in flight trees.

In comparison to previous year, the beetle stages during 2005 were very low, where almost 6000 stages were excavated during this year in both the sites (Figures 11.25-11.28).




Figures 11.21-11.24 Number of eggs, larvae (upper row), and pupae and adults (lower row) in different months in Gunganagar, Chitwan during 2004/05

Similar pattern of occurrence has been observed in different depths. From both years' findings, it can be concluded that the preferable depth for egg laying and larval occurrence is 10-20 cm and larvae are profusely observed during March-April and July-August. Different stages of beetle were recovered in Gunganagar and Gaindakot research sites during 2005 which are presented in Appendices 11.7 and 11.8 respectively.




Figures 11.25-11.28 Number of eggs, larvae (upper row) and pupae and adults (lower row) in different months in Gaindakot, Nawalparasi during 2004/05.

### 11.4.4 Species prevalence in relation to soil depths during 2003/04

The beetle species showed the modest variation with that of their occurrences in different soil depths. None of the stages were found beyond 40 cm of the soil layer (Table 11.4) thus the 1 $m^2$ sample size employed in the current study was adequate. All the larval, pupal, and adult stages were primarily recovered within first 10-20 cm of the soil layer, followed by 20-30 cm while exceptionally very few larvae are available beyond these depths.

Despite of the rearing efforts, eggs to larvae and larvae to adult stages remained largely un-successful, where considerable numbers of larvae were found dead. This situation subsequently limited to the eventual number of adults, therefore, very few of the adults could preserved into the laboratory. The larvae collected into different seasons from different soil depths and subsequently made as adult beetles in the Gunganagar and Gaindakot research sites is presented in Tables 11.4 and 11.5 respectively.

Table 11.4 Prevalence of beetle species obtained from the biweekly sampling as larvae in different seasons and soil depths in Gunganagar, Chitwan, Nepal since November 2003-December 2004. (Identification was carried out after rearing into adults).

| Sampling period | | Sampling depth (cm) | Beetle species | Number of emerged adults |
|---|---|---|---|---|
| Month | Date | | | |
| November | 9.11.03 | 11-20 | *An. dimidiata* Hope | 2 |
| | | | *Heteronychus* sp. 2 | 1 |
| | | 21-30 | *Heteronychus* sp. 2 | 2 |
| | 26.11.03 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *Heteronychus* sp. 2 | 1 |
| | | 21-30 | *M. affinis* Blanchard | 2 |
| December | 12.12.93 | 11-20 | *An. bilobata* Arrow | 1 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |
| | | 30-40 | *Heteronychus* sp. 2 | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 25.12.03 | 0-10 | *Ad. lasiopygus* Burmeister | 1 |
| | | 11-20 | *Allisonotum* simile Arrow | 2 |
| | | 21-30 | *Heteronychus* sp. 2 | 1 |
| | | | *M. affinis* Blanchard | 2 |
| January | 7.1.04 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 2 |
| | | 30-40 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 21.1.04 | 0-10 | *An. xanthoptera* Blanchard | 1 |
| | | 11-20 | *An. dimidiata* Hope | 2 |
| | | | *M. affinis* Blanchard | 1 |
| | | 21-30 | *M. affinis* Blanchard | 1 |
| February | 3.2.04 | 0-10 | *An. dimidiata* Hope | 2 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | 17.2.04 | 11-20 | *M. affinis* Blanchard | 1 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *An. dimidiata* Hope | 1 |
| | | 30-40 | *An. xanthoptera* Blanchard | 2 |
| | | | *M. affinis* Blanchard | 2 |
| March | 3.3.04 | 0-10 | *Ad. versutus* Horald | 2 |
| | | | *An. dimidiata* Hope | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| | 15.3.04 | 0-10 | *Ad. versutus* Horald | 2 |
| | | 11-20 | *M. affinis* Blanchard | 1 |
| | | | *Ad. lasiopygus* Burmeister | 1 |
| | 30.3.04 | 0-10 | *M. affinis* Blanchard | 1 |
| | | | *An. xanthoptera* Blanchard | 2 |
| | | 11-20 | *Ad. versutus* Horald | 2 |
| April | 14.4.04 | 0-10 | *M. cardoni* Brenske | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *Ad. versutus* Horald | 2 |
| | 30.4.04 | 0-10 | *An. dimidiata* Hope | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | | | *Al.simile* Arrow | 1 |

| | | | | |
|---|---|---|---|---|
| May | 13.5.04 | 0-10 | *Anomala* sp. n. 2 | 2 |
| | | | *An. dimidiata* Hope | 2 |
| | | 11-20 | *Mimela silguria* Arrow | 2 |
| | | | *M. affinis* Blanchard | 1 |
| | | | *Ad. lasiopygus* Burmeister | 1 |
| | 26.5.04 | 0-10 | *Anomala sp. n. 2* | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 11-20 | *An. dimidiata* Hope | 1 |
| | | | *M. silguria* Arrow | 2 |
| | | 21-30 | *M. affinis* Blanchard | 2 |
| | | | *Al. simile* Arrow | 1 |
| June | 10.6.04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | | *An. xanthoptera* Blanchard | 1 |
| | | | *Anomala* sp. n. 2 | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *Idionychus excisa* Arrow | 1 |
| | | | *H. liioderes* F. | 2 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *Sophrops* sp. 1 | 2 |
| | 24.6.04 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *Sophrops* sp. 1 | 1 |
| | | | *An. xanthoptera* Blanchard | 1 |
| | | 21-30 | *I. excisa* Arrow | 1 |
| July | 7.7.04 | 0-10 | *H. seticollis* Moser | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 11-20 | *I. excisa* Arrow | 1 |
| | | | Dung beetles | 5 |
| | 21.7.04 | 0-10 | *Sophrops* sp. 1 | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 11-20 | Dung beetles | 4 |
| August | 7.8-04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | | *H. liioderes* F. | 1 |
| | 19.8.04 | 0-10 | *Sophrops* sp. 1 | 1 |
| | | | *An. xanthoptera* Blanchard | 1 |
| September | 2.9.04 | 0-10 | *Al. simile* Arrow | 1 |
| | | 11-20 | *Mimela bicolor* Hope | 2 |
| | 16.9.04 | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *H. nigricolis* F. | 1 |
| | 30.9.04 | | *Mimela bicolor* Hope | 1 |
| October | 13.10.04 | 0-10 | *Ad. versutus* Harold | 1 |
| | 27.10.04 | 11-20 | *Ad. versutus* Harold | 2 |
| | | | *Heteronychus* sp. 2 | 1 |
| November | 10.11-04 | 11-20 | *An. dimidiata* Hope | 2 |
| | | | *Heteronychus* sp. 2 | 1 |
| | | 21-30 | *Al. simile* Arrow | 1 |
| | 23.12.04 | 11-20 | *Heteronychus* sp. 2 | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 21-30 | *I. excisa* Arrow | 1 |
| December | 8.12.04 | 11-20 | *Heteronychus* sp. 2 | 1 |
| | | 21-30 | *H. seticollis* Moser | 2 |
| | 22.12.04 | 11-20 | *An. bilobata* Arrow | 1 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |

The occurrences of the beetle species in Gunganagar in different soil depths in 2003/04 clearly showed the wider variation of beetle species, where *M. affinis* showed the highest (25) frequency of occurrences followed by *Ad. lasiopygus* (10) and *An. dimidiata* (9). Beside these species other beetles species such as *Heteronychus* sp n. 2 (8), *Ad. versutus* (7), *An. xanthoptera* (6) and *A. simile* (5) were found in a considerable extent. Looking into the abundances across the soil depths, *M. affinis* is available throughout the whole season of winter through summer months and all the depths ranging from 10-40 cm. More number of beetle species was found in uppermost soil layers during March-April compared to winter months. During winter periods, most of them were concentrated below than 15 cm and in some cases up to 40 cm depths (Table 11.4). The dung beetles (*Coprius indicus*), however, no damaging species their occurrence frequently confused in the study. Their presentation in these tables is merely to present an overview of the occurrence of the beetle in the study sites.

The biweekly sampling in Gaindakot showed no more differences in the abundance of the beetle species when comparing with former site. Similar to previous site, *M. affinis* ranked the highest frequency of occurrence (19) followed by *H. liodere*s (12) and *Sophrops* sp n. 5 (9). In addition to these beetles other species such as *Anomala* and *Adoretus* occurred as the dominant species in both the study site. They were abundant more or less throughout the year from summer months to the winter months, whereas, *Heteronychus* and few other species such as *Al. simile* are more common during winter season (Table 11.5).

Table 11.5 Prevalence of beetle species obtained from the biweekly sampling as larvae in different seasons and soil depths in Gaindakot, Nawalparasi, Nepal since November 2003-December 2004. (Identification was carried out after rearing into adults).

| Sampling period | | Sampling depth (cm) | Beetle species | Number of emerged adults |
|---|---|---|---|---|
| Month | Date | | | |
| November | 10.11.03 | 11-20 | *An. dimidiata* Hope | 1 |
| | | 21-30 | *H. liodere*s F | 1 |
| | 24.11.03 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 31-40 | *Anomala* sp. n. 2 | 1 |
| December | 8.12.93 | 21-30 | *Ad. versutus* Harold | 2 |
| | | 31-40 | *Ad. lasiopygus* Burmeister | 1 |
| | 23.12.03 | 11-20 | *M. affinis* Blanchard | 1 |
| | | | *Ad. versutus* Harold | 1 |
| | | 21-30 | *Sophrops* spec. 5 | 1 |
| | | | *H. liodere*s F. | 2 |
| January | 5.1.04 | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *An. dimidiata* Hope | 1 |
| | | 31-40 | *Ad. versutus* Harold | 1 |
| | 19.1.04 | 11-20 | *M. cardoni* Brenske | 1 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 2 |
| February | 2.2.04 | 11-20 | *Sophrops* spec. 5 | 1 |
| | | | *M. cardoni* Brenske | 2 |
| | | 31-40 | *Ad. versutus* Harold | 1 |
| | 16.2.04 | 11-20 | *M. affinis* Blanchard | 2 |
| | | 21-30 | *H. liodere*s F | 2 |

| | | | | |
|---|---|---|---|---|
| March | 1.3.04 | 0-10 | *Ad. versutus* Harold | 2 |
| | | | *M. affinis* Blanchard | 2 |
| | | 11-20 | *An. dimidiata* Hope | 1 |
| | | | *Anomala* sp. n. 2 | 1 |
| | 24.3.04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | | *H. seticollis* Moser | 1 |
| | | 11-20 | *Sophrops* spec. 5 | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 29.3.04 | 0-10 | *An. dimidiata* Hope | 1 |
| | | | *H. lioderes* F. | 2 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| April | 13.4.04 | 0-10 | *Schizonychia fuscescens* | 1 |
| | | | Blanchard | 2 |
| | | | *H. seticollis* Moser | |
| | | 11-20 | *An. dimidiata* Hope | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 27.4.04 | 0-10 | *M. cardoni* Brenske | 1 |
| | | | *Sophrops* spec. 5 | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *Anomala* sp. n. 2 | 1 |
| May | 15.5.04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | 11-20 | *S. fuscescens* Blanchard | 1 |
| | | | *H. seticollis* Moser | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 2 |
| | 26.5.04 | 0-10 | *An. xanthoptera* Blanchard | 2 |
| | | | *M. cardoni* Brenske | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *H. lioderes* F. | 2 |
| | | | *Sophrops* spec. 5 | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 2 |
| June | 7.6.04 | 0-10 | *Sophrops* spec. 5 | 1 |
| | | | *Al. simile* Arrow | 1 |
| | | 11-20 | *A. xanthoptera* Blanchard | 2 |
| | | 21-30 | *M. affinis* Blanchard | 1 |
| | 21.6.04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | | *H. lioderes* F. | 2 |
| | | 11-20 | *An. xanthoptera* Blanchard | 2 |
| | | | *Al. simile* Arrow | 1 |
| | | 21-30 | *M. cardoni* Brenske | 1 |
| | | | *M. affinis* Blanchard | 1 |
| July | 5.7.04 | 0-10 | *M. affinis* Blanchard | 2 |
| | | | *Al. simile* Arrow | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 2 |
| | | | *Al. simile* Arrow | 2 |
| | 19.7.04 | 0-10 | *Sophrops* spec. 5 | 1 |
| | | | *H. lioderes* F | 1 |
| | | | *Al. simile* Arrow | 1 |
| | | 21-30 | *H. lioderes* F | 1 |
| August | 2.8-04 | 0-10 | *Sophrops* spec. 5 | 1 |
| | | | *H. lioderes* F | 1 |
| | 17.8.04 | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *H. lioderes* F | 1 |

| | | | | |
|---|---|---|---|---|
| | 31.8.04 | 0-10 | *Al. simile* Arrow | 1 |
| September | 13.9.04 | 11-20 | *M. affinis* Blanchard | 2 |
| | | 21-30 | *Anomala* sp. n.2 | 1 |
| | 28.9.04 | 11-20 | *An. dimidiata* Hope | 2 |
| October | 13.10.04 | 11-20 | *Heteronychus* sp. n.2 | 1 |
| | 27.10.04 | 21-30 | *H. lioderes* F | 2 |
| November | 11.11-04 | 11-20 | *An. dimidiata* Hope | 2 |
| | 25.12.04 | 21-30 | *M. affinis* Blanchard | 2 |
| | | | *H. lioderes* F | 2 |
| December | 6.12.04 | 11-20 | *Anomala* sp. n.2 | 1 |
| | | | *Sophrops* spec. 5 | 1 |
| | 22.12.04 | 21-30 | *An. dimidiata* Hope | 1 |

Some few species were also found at deeper depth up to 40 cm. Looking into the abundance, *Maladera* occurred one of the cosmopolite species across the study site. Furthermore, this study has convincingly showed the larger occurrence of the genus, *Anomala* in aggregate in both the study sites.

### 11.4.5 Species prevalence in relation to soil depths during 2004/2005

Following major species were found prevalent from the sampling conducted over the period of 2005 in Gunganagar and Gaindakot research sites, which is presented in Tables 11.6 and 11.7 respectively.

Table 11.6 Prevalence of beetle species obtained from the biweekly sampling as larvae in different seasons and soil depths in Gunganagar, Chitawan, Nepal since January-November 2005. (Identification was carried out after rearing into adults).

| Sampling period | | Sampling depth (cm) | Beetle species | Number of emerged adults |
|---|---|---|---|---|
| Month | Date | | | |
| January | 6.1.05 | 0-10 | *An. dimidiata* Hope | 2 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 2 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 2 |
| | | | *M. affinis* Blanchard | 1 |
| | 23.1.05 | 0-10 | *An. dimidiata* Hope | 2 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *Ad. versutus* Harold | 1 |
| | | 21-30 | *M. affinis* Blanchard | 1 |
| February | 6.2.05 | 0-10 | *An. dimidiata* Hope | 2 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | 21.2.05 | 0-10 | *M. affinis* Blanchard | 2 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 2 |
| March | 5.3.05 | 0-10 | *An. dimidiata* Hope | 2 |
| | | | *Ad. versutus* Harold | 1 |
| | | | *Ad. lasiopygus* Burmeister | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| | | | *Sophrops* spec. 5 | 1 |
| | 17.3.05 | 0-10 | *An. dimidiata* Hope | 1 |

| | | | | |
|---|---|---|---|---|
| | | | *Ad. versutus* Harold | 2 |
| | | 11-20 | *Sophrops* spec. 5 | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 31.3.05 | 0-10 | *An. dimidiata* Hope | 1 |
| | | | *Ad. versutus* Harold | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| April | 15.4.05 | 0-10 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *M. affinis* Blanchard | 2 |
| | 28.4.05 | 0-10 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *Ad. versutus* Harold | 1 |
| | | | *M. affinis* Blanchard | 1 |
| May | 12.5.05 | 0-10 | *An. xanthoptera* Blanchard | 1 |
| | | | *Anomala* sp. n. 2 | 1 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 1 |
| | 26.5.05 | 0-10 | *An. xanthoptera* Blanchard | 1 |
| | | | *Anomala* sp. n. 2 | 2 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 1 |
| June | 9.6.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | | *I. excisa* Arrow | 1 |
| | | 11-20 | *M. affinis* Blanchard | 1 |
| | | | *Ad. lasiopygus* Burmeister | 1 |
| | | 21-30 | *Ad. versutus* Harold | 2 |
| | | | *An. xanthoptera* Blanchard | 1 |
| | 23.6.05 | 0-10 | *I. excisa* Arrow | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | 11-20 | *M. affinis* Blanchard | 1 |
| | | | *Ad. versutus* Harold | 1 |
| | | 21-30 | *An. xanthoptera* Blanchard | 1 |
| July | 7.7.05 | 0-10 | *H. seticollis* Moser | 1 |
| | | | *I. excisa* Arrow | 1 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *H. liioderes* | 1 |
| | 21.7.05 | 0-10 | *H. seticollis* Moser | 2 |
| | | | *I. excisa Arrow* | 2 |
| | | 21-30 | *Ad. lasiopygus* Burmeister | 1 |
| | | | *Al. simile* Arrow | 1 |
| August | 4.8.05 | 0-10 | *H. liioderes* F. | 1 |
| | | | *I. excisa* Arrow | 5 |
| | | | *M. affinis* Blanchard | 1 |
| | | | *Ad. lasiopygus* Burmeister | 1 |
| | 17.8.05 | 0-10 | *Heteronychus* sp n. 2 | 1 |
| | | | *M. affinis* Blanchard | 1 |
| | | | *Adoretus* sp n. 9 | 1 |
| September | 1.9.05 | 0-10 | *Al. simile* Arrow | 1 |
| | | 11-20 | *M. affinis* Blanchard | 1 |
| | 29.9.05 | 11-20 | *Heteronychus* sp. 2 | 1 |
| October | 27.10.05 | 0-10 | *Heteronychus* sp. 2 | 1 |
| November | All larvae, could not been identified | | | |

Table 11.6 has clearly showed the occurrence of different species of beetles in different depths, where a total of fourteen different beetle species were found in the sampling area. Among them, *M. affinis* was among the highest frequencies (17) followed by *Ad. lasiopygus* (12) and *Ad. versutus* (9). *An. xanthoptera* and *An. dimidiata* found in modest frequencies, whereas, most of the other species were found very infrequently i.e. within 2-5 times of the sampling.

Looking into the depths, none of the species were found below than 30 cm soils and even shallow surface in summer months such as mostly 0-10 cm, however, some of the beetle species such as *M. affinis* and *Ad. lasiopygus* are found up to 30 cm depth. In the same way, some of the beetle such as *Holotrichia, Heteronychus, Adoretus* and *Idionychus* are found common during maize growing seasons. Very interestingly, the occurrences of *M. affinis* was found round the year starting from January through September, which strongly supports its shorter life cycle and overlapping generations within the same year.

Table 11.7 has clearly shown the highest occurrences of *M. affinis* (10), followed by *Ad. versutus* (9) and *An. dimidiata* and *Sophrops* sp. n. 5 (4). The other species occurred in less frequency mostly less than four. Interestingly, the occurrence of beetle species is found in a similar trend as found in previous site, where *M. affinis* recorded the highest occurrence followed by the different species of *Adoretus* although total number of beetle species were comparatively less in this year. Looking into the distribution of beetle species in different soil depths, *M. affinis* were found in shallow depths (0-10 cm) through the deeper depths (21-30 cm).

Table 11.7 Prevalence of beetle species obtained from the biweekly sampling as larvae in different seasons and soil depths in Gaindakot, Nawalparasi, Nepal since January-November 2005. (Identification was carried out after rearing into adults).

| Sampling period | | Sampling depth (cm) | Beetle species | Number of emerged adults |
|---|---|---|---|---|
| Month | Date | | | |
| January | 3.1.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *An. dimidiata* Hope | 1 |
| | | 21-30 | *Ad. versutus* Harold | 1 |
| | 20.1.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *An. dimidiata* Hope | 1 |
| | | 21-30 | *Ad. versutus* Harold | 1 |
| February | 4.2.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | | *Sophrops* sps. 5 | 1 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | | 21-30 | *Ad. versutus* Harold | 1 |
| | 20.2.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | | *Sophrops* sps. 5 | 1 |
| | | 11-20 | *Anomala* sp n. 2 | 1 |
| | | 21-30 | *Ad. versutus* Harold | 1 |
| March | 1.3.05 | 0-10 | *Ad. versutus* Harold | 1 |
| | | | *An. dimidiata* Hope | 1 |
| | | 21-30 | *An. varicolor* Gyllenhal | 1 |
| | 15.3.05 | 0-10 | *Ad. versutus* Harold | 1 |

| | | | | |
|---|---|---|---|---|
| | | | *An. dimidiata* Hope | 1 |
| | | 21-30 | *Anomala* sp. n. 2 | 1 |
| | 29.3.05 | 0-10 | *M. affinis* Blanchard | 2 |
| April | 12.4.05 | 0-10 | *S. fuscescens* Blanchard | 1 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | 26.4.05 | 0-10 | *S. fuscescens* Blanchard | 1 |
| | | 11-20 | *Ad. lasiopygus* Burmeister | 1 |
| May | 10.5.05 | 0-10 | *M. cardoni* Brenske | 1 |
| | | 11-20 | *H. seticollis* Moser | 1 |
| | 24.5.05 | 0-10 | *M. cardoni* Brenske | 1 |
| | | 11-20 | *H. seticollis* Moser | 1 |
| June | 7.6.05 | 0-10 | *Sophrops* sp. 5 | 1 |
| | | 11-20 | *Al. simile* Arrow | 1 |
| | 21.6.05 | 0-10 | *Sophrops* sp . 5 | 1 |
| | | 11-20 | *Al. simile* Arrow | 1 |
| July | 5.7.05 | 0-10 | *Ad. lasiopygus* Burmeister | 1 |
| | | 11-20 | *Ad. versutus* Harold | 1 |
| | 19.7.05 | All larvae could not identified | | |
| August | 2.08.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *M. affinis* Blanchard | 1 |
| | 16.8.05 | 0-10 | *M. affinis* Blanchard | 2 |
| | | 11-20 | *A. simile* Arrow | 1 |
| | 30.8.05 | 0-10 | *M. affinis* Blanchard | 1 |
| | | 11-20 | *M. affinis* Blanchard | 2 |
| September | 13.9.05 | All larvae could not identified | | |
| October | 27.9.05 | All larvae could not identified | | |
| November | 22.10.05 | All larvae could not identified | | |

In the same way, unlike other beetle species these species were found round the year from winter to summer months, probably due to shorter life cycle or many overlapping generations. In this site, some of the different species of beetle such as *S. fuscescens* were also found involved mostly in the upper layer and exceptionally only in the month of April. The major characteristics and virtual figures of the major beetle species is presented in Appendix 11.9.

## 11.5 DISCUSSION

### 11.5.1 Natural vegetation for beetle preference

Variation of the beetle species in the study area could be due to the availability of various species of the plants as well as agricultural crops. *An. dimidiata* harboring large number of plant species compared to other species could possibly be due to frequent occurrences. This situation might have necessitated them to accept the plant species when no other preferable host crops are available whenever they prefer for mating, food, sheltering etc.

In the current study, all the white grub species were found abundant in both cultivated as well as non-cultivated areas, thus females appear to be selecting vegetation emerging from disturbed soil. The flight season occurs during intensive land preparation which is also

launched by the rains. Cultivated fields in both the study sites are not bare for long, as the rains, weeds and crops grow rapidly during planting season, where herbicide application also does not exists. Various white grub species have been found to prefer to oviposit in fields with young plants rather than bare soil or in areas with older plants. Similar finding was reported by King (1985), where various species preferred grass and weeds to bare soil, as young larvae need plant roots to survive.

The feeding preference of white grub species showed *An. dimidiata* usually prefer tall plants such as *G. pinnata, L. chinensis* and other fodder trees, while *H. nigricollis* prefer short vegetation such as hedges of ornamental importance. The preference of *Ad. lasiopygus* is different than former two species and it prefers of involving in the maize seedling. And, this problem is more intense during hot dry spell. Similar finding was reported by Lopez (1930) where, *Leucopholis irrorata* preferred to oviposit in fallowed uncultivated fields with short grassy vegetation rather than standing sugarcane field. He observed most white grub adults flew within 3 m of the ground thus dense vegetation blocked their lateral movement. This study has indicated possible opportunity of using standing field crops such as sugarcane as a physical barrier to adult beetle flight of some species. In fact, windbreaks composed of tall vegetation have been proposed as a control method for white grub (King, 1985). *An. dimidiata*, however, selects tall vegetation for mating and oviposition sites. Several scientists showed that prolonged larval duration when the host crops are unsuitable, in contrast larval development, however, is more rapid in some plant species than others (Cherry and Alisopp, 1991).

### 11.5.2 Population density of white grubs in the soil

There exist correlation between hatching of the eggs and larval occurrence where the egg as well as larval stages are common nearly round the year. In general, the density of different stages of beetles in the research sites is rather low, however, when compared with censuses taken in other countries on improved pastures or sugarcane. In Mauritius, Moutia and Mamet (1946) reported the lowest densities of larvae being $8/m^2$ to the highest densities of $300/m^2$. Similarly, in the UK, *Phyllopertha horticola* and *Hoplia phllanthus* attained high densities in pasture, together reaching $235/m^2$ (Raw, 1951). The lower densities encountered in the study sites may be a result of the lower nutritional value of the native grasses growing on eroded and nutrient depleted acidic soils in low organic matter. The low larval population especially during winter months indicates the diapousing stages and vice versa in summer season. The possible reason of the larval abundance during summer season might have influenced by the climatic factors, species involvement, availability of the host crops, soil properties etc. Moreover, unfavorable weather seems one of the decisive factors influencing the flight of the adult beetles and consequently the abundance of white grubs in any locality.

The limited movement of larvae mainly in localized upper soil layer, suggest that the female must place her eggs in an environment most suited to young grubs. L3 can move somehow deeper layer such as 22-34 cm laterally per day in the soil (Veeresh, 1997), thus they can actively seek new sources of food. The L1 and L2, however, would be less capable of such ranges due to their smaller size. White grubs thrive particularly in locations dominated by grasses with highest densities occurring in perennial crops such as sugarcane or forages compared to annual crops such as maize or upland rice (Wilson, 1969). In the study sites of

Gunganagar and Gaindakot, sugarcane or improved pasture do not exists except only in few plots, but wild perennial grasses are common along with maize crops twice a year.

### 11.5.3 Prevalence of white grub species in the soil

The occurrence of large number of species indicates the involvement of various species of beetles within a smaller geographical area of Nepal. Similar findings are reported by several scientists, where over a dozen species of economic importance occur together in both Java (Kalshoven, 1981) and Queensland (Illingworth and Dood, 1921) fourteen species in Karnataka India (Veeresh, 1977), and dozens of species in Central America (King, 1985) to name a few regions. In fact, however, only one or two species tend to dominate. In the study site near about a dozen of species, 2-3 dominant and rest of the species are of minor importance. The repeated occurrence of similar species over the entire recording period overshadows the doubts that if sampling were extended to other areas in the Gunganagar and Gaindakot conditions, more white grub species would be encountered.

The association of larvae in the crop roots and root zone area supports that all the species are pests. Very noticeably *H. liioderes* involve on maize seedlings. It was assumed that all of them are attracted towards the crop roots, although pot studies should be undertaken for verification. It is curious, *Maladera* are dominantly present in Chitwan conditions compared to Nawalparasi even though the sites are not very far from each other. Several scientists have reported that the trends of beetle distribution which is normally limited to a country or region with a country. The exception seems to *M. melolontha* L. which is distributed throughout Europe and parts of China (Litsinger *et al.* 2002) and few nearest kin of *Maladera indica* in Nepal through light traps (Chapter 9).

### 11.5.4 Number of white grubs generations

White grubs worldwide exhibit a wide range of life cycle duration. Because of the lack of knowledge on the identification of the larvae, the life cycle of all the species could not been studied except *M. affinis* (Chapter 10). From the population dynamics study, all the species exhibited annual cycles as the larval populations (especially of L3) had prominent peaks at least in two seasons and regular occurrences round the year. According to the King (1984), two year life cycle may occur in locations with a longer dry season more than 4-6 months, however, which may not be the case in the present study sites. Ritcher (1958), observed among species, that those in more northerly (colder) areas take 2-3 years to develop, whereas 1-2 years is common in more southern locations. Several workers have reported that even numbered year cycle appears to be independent of rainfall pattern which subsequently affect on the year of dominance even in the same locality (Uichanco, 1936). TAI (1986) found that some individuals of *Anomala cupripes* matured in six months while others took one year. She further mentioned the difference was attributed to the speed of L1 and L2 development. It is difficult to distinguish between true multiple generations and delayed development. This can be the result of environmental or genetic causes. Development of some individuals within a species can be prolonged due to late oviposition, environmental factors, inadequate nutrition, dormancy or genetic differences in physiologies (Litsinger *et al.* 2002).

Numbers of factors are responsible for the development of generations of white grubs. Remarkably, sporadic rainfall can stagger adult emergence to explain the appearance of stages

out of synchrony. Similarly, egg may remain without hatching for considerable period of time because of unfavorable temperature, and larvae can arrest development during any part of the year if nutritional requirements are not met. Quality of food is another important parameter that affect rate of development. Veeresh (1977) reported that *Holotrichia serratea* L1 can remain for up to 110 days in the laboratory in the egg cell if moisture is minimal but not saturated. He further reported, adult emergence from soil can be protected over several months. Egg laying was also not continuous, as females need better oviposition site. The consequence of delayed oviposition and development strongly suggest the possibility of over lapping stages in the field giving the appearance of multiple generations. Thus for those white grub species common in Chitwan and Nawalparasi during their larval stages were found almost year round, as such occurrence could be either due to attempted multiple generations in one year or delayed development as these phenomenon could not be distinguished in current study.

### 11.5.5 Soil depth and microclimate relationships

All of the life stages of white grubs spend at least a part of their life cycle in the soil. It was noted that each species of white grubs in the study sites had preferred depths. The depth to which they descend is a product of the needs of the life stage in relation to risks of desiccation or drowning manifested by weather and prevailing climate. Farrell (1972) mentioned there is a trade-off between soil depth and moisture relation and it is species and stage dependent. Soil moisture is reported to be crucial for the hatching of the eggs and survival of the rest of the stages. TAl (1986) studies the effect of a range of soil moisture (10-40% field capacity) on egg and larval survival found moisture at 20-30% field capacity optimal for egg survival. Similar to these studies, the lower number of eggs especially in upper layer might have influenced due to soil moisture. On the other hand, heavy rainfall for prolonged period could also be detrimental for larval survival.

Normally, white grubs descend in the soil profile to lower depths when they are about to pupate or enter a resting stage and the selected depth appears to be a function of soil type, body size, and prevailing climate. Those that dig deeper tend to be in lighter soils, drier or colder climates, and are larger in size. Similar observation was noticed while obtaining the larger sizes of larvae of *Xylotrupes gideon* in Parbat District with that of the smaller larvae of *Maladera, Anomala* and other tropical species where the former types of larvae tend to remain in deeper layer sometimes beyond 50 cm deep (Field observation). However, in general, preferred soil depth seemed to be independent with respect to larval size of the species common in Chitwan and Nawalparasi Districts. The relatively shallow depths in which the white grubs in these study sites were recovered is a reflection of the low moisture holding by the highly friable soils making tunneling relatively easy, shallow crop roots thus the mortality from desiccation was likely to be minimal compared to other locations.

### 11.5.6 Adult abundance in flight trees

White grubs are well known for their synchronized mass emergence flights in response to early rains since it makes easy for digging to adults. This could be one of the reasons for the abundance of adult beetles during pre-monsoon and rainy season where large numbers of *An. dimidiata* were sparsely distributed in the plant species at the study sites. Based on the experimental results in Australia, Litsinger *et al.* (2002) reported the generalized flight

behavior of adults which are common to most of the adult beetles. According to them, the first type of flight behaviour is indicative of *Anomala consanguineous, A. parvulus, Lepidiota picticollis,* and *Leucopholis crinita*. They are reported to be fully sexually mature upon emergence and do not feed nor seek flight trees. Males fly to the emergence hole to mate with newly emerged females who then lay their eggs nearby. In our study, the recovered beetles were not found of this kind of group.

The second type is exemplified by *L. negabria, L. frenchi,* and *Phyllopertha horticola* in which adults fly to trees to mate. In this case, the adults are not necessarily sexually mature upon emergence, however, but are ready to mate. After mating, females then make a second short distance flight to enter the ground to oviposit, even entering the same emergence hole to oviposit. Lopez (1931) mentioned eggs are laid in groups of up to fourteen eggs, thus the beetle under this group make several tunnels to oviposit all their egg clusters. Mating takes less than 20 minutes and probably occurs between each batch of eggs laid. Some of the beetles found in the present study may fall in this group. The third type is exemplified by *Dermolepida albohirtum* in which adults are immature upon emergence, therefore, both sexes fly to the nearest trees or tall vegetation irrespective of wind direction. After mating in the tree and egg maturation, females fly to the field to oviposit underground and may spend more than a day depositing a batch of eggs deep in the soil. After the first batch of eggs females can return to trees and repeat the cycle one or more times. It is reported that how many times this can occur but possibly up to three times. In line of the characteristic of these beetles, our study indicated that common tropical species fall under first group; however, *An. dimidiata* probably fall under second category since mating was seen during the day in the tree.

Adult host range is generally very broad in the species that have been extensively studied elsewhere. In some cases it seemed to relative since the host preferences sometimes does not follow the definite trend since it is more dependent on the availability of the hosts. Several workers have reported similar findings in several parts of the world. Kalshoven (1981) reported *An. destructor* in Java, initially preferred to legumes but also attacked fruit trees and garden plants. In the same way, *H. serrata* in India was recorded on seventeen host plants, preferring neem and *Acacia arabica* (Veeresh, 1977), however some species were not preferred in field trails (Rai *et al.* 1969). Present study indicated the flight trees belonged to wide host range, but botanical affinity provided few clues, as even within a genus there seems a range of preferences among closely related species. Beetles were also seen involved in bamboo trees, however, leaf damage was not noticed, probably such plant might have utilized only for mating purpose. In general, one definite trend has observed where nearly all species preferred newly developed flush which could be due to availability of more nutritious food than older leaves that contain secondary chemicals.

### 11.5.7 Species ecological preferences

White grubs are favored by light sandy soils, fibrous rooted plants, and high organic matter content. This could be probably one reason of more larvae found in Gunganagar and Gaindakot study site. Throughout observation they were not abundant in waterlogged, compacted, stony soils, or lands lacking vegetation. Raw (1951) found that, *P. horticola* preferred side slopes of hilly lands, high rainfall, light soil, and grass lands that are poor quality permanent pasture. Other species show differential preferences to physical parameters (Hassan and Hilditch, 1976). It is to be expected that each of the white grub species recovered

in the study sites would also express different adaptations. The study has convincingly showed dominant white grub species in these localities have no even year life cycle with larger population of allochronic (odd years) or having an annual life cycle. The distribution of beetle species in similar pattern in both of the research site might have favored due to environmental factors coupled with hosts crops and soil parameters. Because of the tropical environment most of the beetle species might have shorter life.

## 11.6 CONCLUSIONS

The vulnerable period for maize and other bari land based crops to white grub damage occurs during the seedling stage from April-June. The greatest damage is done by the L3 stage and its densities are especially important to note as one larva can kill a young plant (Litsinger, 1993) where a seedling death would result a greatest loss than adult plants. The occurrence of larva in two peaks in a present study suggests farmer may focus control measure either with biological products twice a year instead of once per year. If such measures were to be adopted targeting the L1 stage is most economical compared to advance stages since the efficiency can occur at very low dosages. Whereas, in another hand control measures can be directed at even numbered year, if the grub species have biannual life cycle this may be the case of high hills of Nepal such as Sindhupalchowk and Parbat Districts. Chandler *et al.* (1993) noted that sugarcane growers in Queensland use chlorpyrifos against white grubs and achieved moderate success. In general, white grubs larva abundant in uppermost soil layer, often killed unintentionally during agricultural operations such as plowing, harrowing, digging etc. Most (75%) of the larval stages are within the top 20 cm of soil on average. Farmers therefore, are suggested to perform summer as well as winter plowing at little bit dipper depths using tractors which further allows higher chances of destruction. In addition, use of decomposed farm yard manure coupled with winter plowing would be beneficial in minimizing the grubs. In the same way, there seems greater prospect of augmenting as biological pesticides of entompathogenic fungi such as *M. anisopliae* and *B. bassiana* as an important component for sustainable control of white grubs' since the host population (larva) is available round the year. Looking into the soil temperature, the application of microbial products would be more effective in winter season as compared to the summer season where soil temperature in later period reaches higher. In general, understanding of the pest biology is an important aspect for planning of the pest management strategies. This is still imperative where no systematic approaches such as study of the pest dynamics etc for pest control are often lacking like Nepal.

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## Appendices

**Appendix 11.1 Monthly average rainfalls, humidity, atmospheric temperature and soil temperature of the experimental sites during 2003-2005.**

| Year | | Temperature (°C) | | | | Relative humidity (%) | Total rainfall (mm) |
|---|---|---|---|---|---|---|---|
| | | Atmospheric temperature | | Soil temperature | | | |
| | Month | Maximum | Minimum | Maximum | Minimum | | |
| 2003 | January | 21.3 | 9.09 | 19.3 | 13.1 | 98.2 | 42.4 |
| | February | 26.3 | 11.1 | 22.6 | 14.3 | 96.9 | 54.0 |
| | March | 34.1 | 15.5 | 26.4 | 21.8 | 84 | 85.0 |
| | April | 33.9 | 21.3 | 31.7 | 21.6 | 74.2 | 120.6 |
| | May | 34.2 | 23.4 | 32.9 | 26.2 | 78.0 | 132.2 |
| | June | 34.6 | 23.8 | 32.6 | 29.8 | 78.2 | 135.6 |
| | July | 32.3 | | 32.8 | 28.9 | 87.3 | 201.5 |
| | August | 32.1 | 24.1 | 31.3 | 32.4 | 84.7 | 264.1 |
| | September | 32.9 | 25.3 | 31.9 | 27.6 | 88.9 | 227.3 |
| | October | 30.8 | 20.3 | 29.5 | 23.2 | 86.1 | 76.2 |
| | November | 29.0 | 14.1 | 24.2 | 17.3 | 92.5 | 31.0 |
| | December | 23.8 | 9.9 | 20.6 | 16.5 | 98.0 | 0.0 |
| 2004 | January | 21.1 | 9.07 | 1.3 | 13.8 | 98.3 | 62.7 |
| | February | 26.7 | 10.3 | 21.7 | 15.2 | 97.9 | 0.0 |
| | March | 33.2 | 15.7 | 27.9 | 21.2 | 82 | 0.0 |
| | April | 33.4 | 20.2 | 30.6 | 24.8 | 75.3 | 180.2 |
| | May | 34.8 | 22.6 | 33.3 | 27.8 | 75.06 | 111.4 |
| | June | 34.6 | 24.4 | 33.2 | 29 | 79.8 | 15.8 |
| | July | 30.1 | 25.3 | 31.9 | 29.2 | 88 | 15.5 |
| | August | 34.4 | 25.8 | 33.4 | 30.2 | 85.6 | 214.3 |
| | September | 33.3 | 24.5 | 32.7 | 28.7 | 87.5 | 417.7 |
| | October | 31.5 | 19.2 | 28.6 | 24.5 | 85.6 | 75.7 |
| | November | 28 | 12.9 | 23.4 | 18.6 | 93.3 | 12.0 |
| | December | 24.7 | 9.7 | 19.5 | 14.8 | 99 | 0.0 |
| 2005 | January | 23.2 | 9.1 | 12.8 | 17.5 | 98.7 | 38.1 |
| | February | 26.7 | 10.3 | 14.4 | 20.5 | 97.9 | 0.0 |
| | March | 31.4 | 14.9 | 19.6 | 25.2 | 95.0 | 38.9 |
| | April | 35.9 | 21.6 | 22.1 | 29.3 | 68.1 | 133.5 |
| | May | 36.2 | 23.4 | 24.0 | 30.4 | 65.5 | 28.8 |
| | June | 36.6 | 25.1 | 26.6 | 31.0 | 71.7 | 133.9 |
| | July | 33.0 | 25.5 | 29.5 | 32.2 | 86.5 | 349.2 |
| | August | 28.4 | 27.3 | 29.2 | 32.3 | 89.3 | 671.1 |
| | September | 34.5 | 25.06 | 29.5 | 32.7 | 84.0 | 148.6 |
| | October | 31.6 | 20.1 | 25.3 | 29.3 | 89.1 | 183.5 |
| | November | 28.15 | 13.02 | 19. 2 | 26.3 | 94.2 | 51.4 |
| | December | 25.05 | 8.82 | 17.9 | 24.9 | 100 | 13.2 |

**Appendix 11.2 Most prevalent plant species preferred by adult beetle of *Anomala dimidiata* and its association* over the recording period in Gunganagar and Gaindakot, 2005.**

| S. N. | Plant species | | Recording duration and number of beetle (n = 10 samplings) | | | | | Total beetles |
|---|---|---|---|---|---|---|---|---|
| | Local Name | Scientific Name | April | May | June | July | August | |
| 1 | Dabdabe | *Garuga pinnata* | 23 | 57 | 85 | 106 | 42 | 313 |
| 2 | Bakaino | *Melia azedarach* | 21 | 41 | 32 | 98 | 36 | 228 |
| 3 | Acalifa | *Acalypha* sp | 0 | 23 | 45 | 102 | 51 | 221 |
| 4 | Rose | *Vinca rosea* | 18 | 21 | 41 | 78 | 43 | 201 |
| 5 | Baharmase | *Hibischus sp* | 15 | 31 | 42 | 54 | 51 | 193 |
| 6 | Maize** | *Zea mays* | 45 | 59 | 87 | 0 | 0 | 191 |
| 7 | Litchi | *Litchi chinensis* | 0 | 17 | 21 | 16 | 0 | 54 |
| 8 | Millet** | *Elusina coracana* | 0 | 0 | 0 | 17 | 26 | 43 |
| 9 | Khanyu | *Ficus sp* | 0 | 9 | 10 | 18 | 2 | 39 |
| 10 | Mango | *Mangifera indica* | 0 | 9 | 7 | 8 | 0 | 24 |

**Appendix 11.3 Most prevalent plant species preferred by adult beetle of *Holotrichia nigricolis* and its association* over the recording period in Gunganagar and Gaindakot, 2005.**

| S.N. | Plant species | | Recording duration and number of beetle (n = 10 samplings) | | | | | Total beetles |
|---|---|---|---|---|---|---|---|---|
| | Local Name | Scientific Name | April | May | June | July | August | |
| 1 | Dabdabe | *Garuga pinnata* | 21 | 33 | 46 | 98 | 0 | 198 |
| 2 | Bakaino | *Melia azedarach* | 0 | 12 | 33 | 56 | 0 | 101 |
| 3 | Acalifa | *Acalifa sp* | 12 | 33 | 0 | 0 | 0 | 45 |
| 4 | Rose | *Vinca rosea* | 0 | 12 | 19 | 10 | 0 | 41 |
| 5 | Baharmase | *Hibischus sp* | 10 | 14 | 2 | 8 | 6 | 40 |
| 6 | Maize** | *Zea mays* | 8 | 5 | 7 | 16 | 0 | 36 |
| 7 | Litchi | *Litchi chinensis* | 0 | 14 | 21 | 6 | 8 | 49 |
| 8 | Millet** | *Elusina coracana* | 0 | 0 | 0 | 24 | 12 | 36 |
| 9 | Khanyu | *Ficus sp* | 0 | 23 | 12 | 40 | 3 | 78 |
| 10 | Mango | *Mangifera indica* | 0 | 6 | 12 | 14 | 8 | 40 |

**Appendix 11.4 Most prevalent plant species preferred by adult beetle of *Adoreturs liioderes* and its association* over the recording period in Gunganagar and Gaindakot, 2005.**

| S.N. | Plant species | | Recording duration and number of beetle (n = 10 samplings) | | | | | Total beetles |
|---|---|---|---|---|---|---|---|---|
| | Local Name | Scientific Name | April | May | June | July | August | |
| 1 | Dabdabe | *Garuga pinnata* | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | Bakaino | *Melia azedarach* | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Acalifa | *Acalifa sp* | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | Rose | *Vinca rosea* | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | Baharmase | *Hibischus sp* | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | Maize** | *Zea mays* | 22 | 45 | 65 | 12 | 0 | 144 |
| 7 | Litchi | *Litchi chinensis* | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | Millet** | *Elusina coracana* | 0 | 0 | 0 | 31 | 19 | 50 |
| 9 | Khanyu | *Ficus sp* | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | Mango | *Mangifera indica* | 0 | 0 | 0 | 0 | 0 | 0 |

**Appendix 11.5 Number of different stages of beetles (eggs, larvae, pupae and adults) in different depth of 10 $m^2$ area with respect to different date of observation (Interaction effect of depth x dates) in Gunganagar, Chitwan, Nepal since November 2003 – December 2004.**

| Recording month | Number of eggs (E), larvae (L), pupae (P) and adults (A) at different depths (cm) in different months of observation | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-10 cm | | | | 11-20 cm | | | | 21-30 cm | | | | 31-40 cm | | | | 41-50 cm | | | | 51-60 cm | | | | 61-70 cm | | | |
| | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A |
| Nov 03 | 8 | 131 | 0 | 0 | 13 | 113 | 5 | 8 | 6 | 133 | 8 | 6 | 13 | 48 | 0 | 3 | 13 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 03 | 0 | 66 | 13 | 0 | 5 | 86 | 3 | 5 | 8 | 105 | 0 | 0 | 0 | 48 | 8 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jan 04 | 0 | 66 | 0 | 0 | 0 | 113 | 0 | 3 | 18 | 91 | 0 | 6 | 0 | 68 | 12 | 0 | 8 | 76 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb 04 | 0 | 76 | 0 | 6 | 0 | 116 | 0 | 5 | 10 | 95 | 0 | 0 | 0 | 48 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar 04 | 13 | 248 | 3 | 0 | 0 | 312 | 3 | 8 | 51 | 183 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr 04 | 3 | 233 | 3 | 3 | 8 | 225 | 3 | 16 | 16 | 100 | 0 | 8 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 04 | 40 | 236 | 0 | 13 | 138 | 221 | 15 | 33 | 33 | 111 | 3 | 0 | 0 | 10 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| June 04 | 0 | 266 | 0 | 0 | 8 | 234 | 0 | 6 | 12 | 126 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| July 04 | 0 | 216 | 5 | 6 | 0 | 175 | 0 | 0 | 0 | 61 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aug 04 | 20 | 231 | 0 | 35 | 0 | 210 | 8 | 13 | 0 | 99 | 12 | 0 | 0 | 23 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep 04 | 0 | 298 | 8 | 5 | 5 | 258 | 8 | 3 | 0 | 138 | 6 | 3 | 10 | 10 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct 04 | 0 | 18 | 3 | 5 | 8 | 146 | 8 | 6 | 0 | 173 | 8 | 5 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nov 04 | 0 | 43 | 8 | 11 | 16 | 113 | 16 | 8 | 8 | 130 | 0 | 5 | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 04 | 0 | 8 | 0 | 0 | 0 | 97 | 8 | 16 | 30 | 110 | 0 | 8 | 13 | 23 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD (5%) | Eggs (15.62),; Larvae (36.43), Pupae (6.41); Adults (6.176) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SEM | Egss (5.62) ; Larvae (13.12); Pupae (2.30); Adults (2.22 ) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CV% | Egg (463) ; Larvae (81 ); Pupae (546.1); Adults (404.6) | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Appendix 11.6 Number of the different stages of beetles (eggs, larvae, pupae and adults) in different depth of 10 $m^2$ area with respect to different date of observation (Interaction effect of depth x dates) in Gaindakot, Nawalparasi, Nepal since November 2003 – December 2004.**

| Recording month | Number of eggs (E), larvae (L), pupae (P) and adults (A) at different depths (cm) in different months of observation | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-10 cm | | | | 11-20 cm | | | | 21-30 cm | | | | 31-40 cm | | | | 41-50 cm | | | | 51-60 cm | | | | 61-70 cm | | | |
| | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A |
| Nov 03 | 3 | 3 | 0 | 0 | 13 | 38 | 18 | 6 | 11 | 81 | 3 | 0 | 10 | 18 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 03 | 0 | 0 | 0 | 0 | 21 | 95 | 0 | 10 | 18 | 98 | 5 | 10 | 28 | 86 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jan 04 | 0 | 41 | 0 | 0 | 3 | 88 | 0 | 3 | 0 | 81 | 0 | 0 | 0 | 83 | 0 | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb 04 | 0 | 73 | 0 | 6 | 0 | 90 | 0 | 10 | 0 | 80 | 0 | 5 | 18 | 46 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar 04 | 0 | 146 | 0 | 10 | 26 | 163 | 25 | 13 | 8 | 113 | 3 | 21 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr 04 | 0 | 181 | 0 | 23 | 35 | 120 | 3 | 23 | 0 | 128 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 04 | 0 | 178 | 8 | 5 | 0 | 130 | 13 | 8 | 0 | 88 | 0 | 6 | 0 | 13 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| June 04 | 0 | 160 | 0 | 26 | 18 | 110 | 5 | 3 | 0 | 53 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| July 04 | 13 | 146 | 13 | 13 | 3 | 213 | 0 | 0 | 0 | 120 | 0 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aug 04 | 0 | 76 | 0 | 3 | 10 | 143 | 0 | 10 | 0 | 117 | 0 | 3 | 0 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep 04 | 5 | 116 | 10 | 18 | 25 | 108 | 3 | 23 | 5 | 115 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct 04 | 0 | 11 | 3 | 18 | 0 | 53 | 21 | 30 | 0 | 96 | 0 | 43 | 0 | 25 | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nov 04 | 0 | 0 | 0 | 18 | 0 | 90 | 13 | 30 | 18 | 85 | 5 | 25 | 18 | 41 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 04 | 0 | 8 | 0 | 13 | 0 | 70 | 28 | 21 | 13 | 116 | 5 | 34 | 15 | 46 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD (5%) | Eggs (9.22),; Larvae (21.90), Pupae (5.032); Adults (7.79) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SEM | Egss (3.32) ; Larvae (7.88); Pupae (1.812); Adults (2.80) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CV% | Egg (42.70) ; Larvae (73.30); Pupae (35.2); Adults (26.50) | | | | | | | | | | | | | | | | | | | | | | | | | | | |

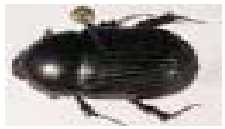
**Appendix 11.7 Number of different stages of beetles (eggs, larvae, pupae and adults) in different depth of 10 $m^2$ area with respect to different date of observation (Interaction effect of depth x dates) in Gunganagar, Chitwan, Nepal since January – November 2005.**

| Recording month | Number of eggs (E), larvae (L), pupae (P) and adults (A) at different depths (cm) in different months of observation | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-10 cm | | | | 11-20 cm | | | | 21-30 cm | | | | 31-40 cm | | | | 41-50 cm | | | | 51-60 cm | | | | 61-70 cm | | | |
| | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A |
| Jan 05 | 5 | 13 | 3 | 0 | 0 | 85 | 0 | 0 | 0 | 97 | 0 | 5 | 0 | 57 | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb 05 | 0 | 30 | 3 | 0 | 0 | 85 | 5 | 0 | 5 | 100 | 0 | 3 | 0 | 77 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar 05 | 0 | 145 | 3 | 6 | 0 | 128 | 4 | 0 | 5 | 75 | 0 | 10 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr 05 | 0 | 100 | 0 | 7 | 10 | 81 | 0 | 15 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 05 | 0 | 120 | 0 | 0 | 15 | 83 | 0 | 13 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| June 05 | 0 | 142 | 0 | 20 | 15 | 91 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| July 05 | 0 | 108 | 3 | 18 | 0 | 70 | 0 | 3 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aug 05 | 0 | 201 | 5 | 7 | 0 | 120 | 4 | 4 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep 05 | 0 | 60 | 0 | 0 | 13 | 62 | 0 | 5 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct 05 | 0 | 111 | 10 | 6 | 30 | 109 | 0 | 13 | 0 | 82 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nov 05 | 0 | 60 | 5 | 20 | 3 | 110 | 17 | 12 | 3 | 92 | 0 | 14 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 05 | 0 | 32 | 6 | 10 | 20 | 91 | 13 | 30 | 22 | 55 | 17 | 31 | 12 | 60 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD (5%) | Eggs (0.7173); Larvae (2.854), Pupae (0.697); Adults (0.789) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SEM | Egss (0.2582); Larvae (1.027); Pupae (0.251); Adults (0.284) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CV% | Egg (645.5) ; Larvae (112.7 ); Pupae (651.2); Adults (394.1) | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Appendix 11.8 Number of different stages of beetles (eggs, larvae, pupae and adults) in different depth of 10 $m^2$ area with respect to different date of observation (Interaction effect of depth x dates) in Gaindakot, Nawalparasi Nepal since January – November 2005.**

| Recording month | Number of eggs (E), larvae (L), pupae (P) and adults (A) at different depths (cm) in different months of observation | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-10 cm | | | | 11-20 cm | | | | 21-30 cm | | | | 31-40 cm | | | | 41-50 cm | | | | 51-60 cm | | | | 61-70 cm | | | |
| | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A | E | L | P | A |
| Jan 05 | 0 | 50 | 0 | 0 | 0 | 49 | 0 | 8 | 4 | 65 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb 05 | 0 | 19 | 5 | 10 | 0 | 69 | 5 | 7 | 0 | 39 | 0 | 0 | 0 | 17 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar 05 | 3 | 10 | 0 | 3 | 0 | 47 | 25 | 4 | 4 | 39 | 15 | 7 | 0 | 5 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr 05 | 0 | 28 | 0 | 2 | 0 | 40 | 25 | 9 | 0 | 26 | 22 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 05 | 0 | 66 | 0 | 0 | 0 | 41 | 0 | 10 | 0 | 3 | 0 | 0 | 0 | 6 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| June 05 | 0 | 55 | 0 | 8 | 0 | 19 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| July 05 | 0 | 65 | 0 | 0 | 0 | 35 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aug 05 | 0 | 142 | 0 | 0 | 0 | 75 | 0 | 6 | 3 | 15 | 0 | 0 | 0 | 12 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep 05 | 0 | 38 | 0 | 0 | 4 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct 05 | 5 | 91 | 5 | 13 | 21 | 87 | 6 | 25 | 3 | 91 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nov 05 | 0 | 47 | 0 | 19 | 16 | 91 | 45 | 25 | 2 | 77 | 12 | 37 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec 05 | 0 | 22 | 0 | 30 | 4 | 99 | 10 | 41 | 6 | 79 | 5 | 65 | 0 | 33 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LSD (5%) | Eggs (0.3894); Larvae (1.813), Pupae (0.509); Adults (0.674) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SEM | Egss (0.1402); Larvae (0.652); Pupae (0.183); Adults (0.242) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CV% | Egg (784.9) ; Larvae (125.9); Pupae (413.7); Adults (244.5) | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Appendix 11.9 Major beetle species recovered during population dynamics study during November 2003-November 2005 in Gunganagar and Gaindakot research sites of Nepal.**

| Beetle species | Major characteristics (References, Arrow, 1917; 1925 and 1935) |
|---|---|
|  *Schizonychia fuscescens* (Melolonthinae) | Posterior spiracles placed in scarcely diverging lines, claws generally fixed and equal. Incase of larvae, anal opening angulate or Y shaped and claws of 3rd leg reduced. |
|  *Holotrichia seticolis* (Melolonthinae) | Raster with irregular setae or with two longitudinal rows of setae. |
|  *Maladera affinis* (Melolonthinae) | Common species in sandy soil in Chitwan condition, typical segment on lateral part of larval head, red colour smaller in size, closed pygidium with the wings. Stipes (maxillae) strongly enlarged. |
|  *Idionychus excisa* (Melolonthinae) | Antennal club with more than 3 antennomeres, labrum symmetric, pronotum wide, frons with a transverse carina, body yellowish brown. The frons are very shiny. Last abdominal tergite densely setose. |
|  *Heteronychus lioderes* (Dynastinae) | Pygidium strongly and uniformly punctured, coarse and confluent. Black above, deep reddish brown beneath and very smooth and shining elongate-oval in shape and not very convex. The pronotum and scutellum are entirely smooth and shinning. The pygidium is very deep and coarse. This is very abundant species and adult becomes more active during November and December. In the larvae, the raster with a pair of robust setae. |
|  *Allisonotum simile* (Dynastinae) | Black, smooth and shining, convex and elongate-oval. The pronotum is strongly punctured and the punctures are dense at the sides. The scutellum is smooth and the elytra are very coarsely and deeply punctured in rows, the apical margins being coarsely and irregularly punctured. Beetle is smaller in size not more than 10-11 mm in length and about 6 mm in breadth. In the larvae, the raster with a pair of robust setae. |

*Phyllagnathus dionysius* (Dynastinae)

Also known as rice cockchafers, mandibles partly visible externally; front coxae transverse, the scutellum is always exposed, small and bluntly triangular, elytra completely cover the abdomen except the pygidium, mandibles not notched externally, sexes dissimilar, male shorter and more globose than female. Head armed with a short, flattened and recurved horn and pronotum excavated in the middle. In female, the pygidium is not very convex and is punctured and thinly clothed with erect hairs. In the larvae, the raster with a pair of robust setae.

*Anomala xanthoptera* (Rutelinae)

Yellow, elytra peculiar pale green, shortly oval, convex and moderately shining, scutellum finely punctured, pygidium finely granulated. Raster with two rows of longitudinal setae. Setae with palidium shorter. Palidium with 18 setae.

*Anomala dimidiata* (Rutelinae)

Apple green, body shape broadly oval, clypeus densely punctured, pygidium moderately transverse. Setae with long palidium which with 30 setae.

*Anomala cantori* (Rutelinae)

Uniform dark coppery above and beneath, Broadly oval, pronotum, scutellum and elytra densely punctured, pygidium is transversely striolated. Setae with palidium long. Palidium with 20 setae.

*Adoretus lasiopygus* (Rutelinae)

Dark brown, pronotum less closely punctured at the sides than in the middle, large pygidium in male. Raster without two longitudinal rows of setae.

*Mimela silguria (Rutelinae)*

Head generally rather broadly ovate, sometimes globaose, head generally rather broad, whit the clypeus short, Antennae 9 jointed

*Chilolaba acuta* (Cetoninae)

Green rose chafers, larvae move ventrally and sometimes dorsoventrally and have well developed hairs on the body. Major pest of flower. Labrum symmetrical, claws cylindrical with numerous setae. $9^{th}$ and $10^{th}$ abdominal segment fused dorsally.

| | | |
|---|---|---|
|  | *Coprius indicus* (Coprinae) | Black, body compact, convex or a little depressed, abdomen very short, femora very thick, the elytra are very strongly sulcate. Maxillae with lacinia and galea are distinctly separated. |
|  | *Anomala bilobata*, (Rutelinae) | Body elongated and cylindrical, head small and deeply punctured, pronotum finely punctured, pygidim rugosely punctured |
|  | *Maladera cardoni* (Melolonthinae) | Found singly and infrequently in profusely in the sandy soils, pygidium narrow, commonest species in Chitwan condition, head anteriorly weekly narrowed, not produced, Metacoxae enlarged, Metatarsus with two claw |

# Chapter 12

# General discussion and conclusions

# Chapter 12

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## 12.1 INTRODUCTION

White grubs are economically important insect pests in Nepal, however, their strategic management is lacking in the country (G. C. and Keller, 2002). Chemical pesticides have been the practical method used by growers for many decades, but their continuous use can no longer be done because of resistance problems, side effects on non-target organisms, surface- and groundwater contamination, residues on food crops etc. Entomopathogenic fungi are one of the best alternatives that regulate many arthropod populations, including several pest species. They are regenerative in the natural environment and resistance build up by the insect pest is less likely. In addition, most of the solely chemical based technologies are likely to be useful for only a few years before the insects build resistance. In this sense, microbial control would be a more sustainable approach for the increased farm production. A variety of strategies have been used successfully to manipulate fungi in biological control programs. Most of the research conducted on fungal pathogens of insects has emphasized the use of these organisms as microbial insecticides. To that end, the bio-control of white grubs as initiated by Helvetas and IAAS, Rampur, is the first attempt in Nepal with an inoculation strategy as defined by Keller (2000) and Eilenberg, *et al.* (2001) using an indigenous fungus, *Metarhizium anisopliae*.

Intelligent use of fungi as biological control agents will require detailed knowledge of their pathobiology, epizootiology, host biology, and interactions with other components of the ecosystems in which they are to be used. Natural disease development and spread are governed by many factors with focus on the ability of the strains to induce epizootics to the host populations. In addition, abiotic environmental factors such as moisture, temperature, solar radiation and soil properties influence whether or not infections can occur. Improvement of our knowledge on the production, application and initiation of epizootics under field conditions is very important to optimise the control strategies of white grubs with *M. anisopliae*. To this end, a series of laboratory and field experiments were conducted to determine the efficacy of indigenous isolates of *M. anisopliae* for white grub control. The aspects of host and pathogen biology, screening of virulent strains for disease development, mass production, environmental factors that allow or restrict disease progression, and the application as a means of biological control strategies in the Nepalese situation are discussed in Chapters three to seven. Chapter nine to eleven are related to host insect determination and the identification of damaging species of white grubs, their phenology and dynamics in relation to microbiological control.

## 12.2 GENERAL DISCUSSION OF THE RESULTS

Entomogenous fungi have a great promise for use as biological control agents against different insects; however, their infectivity is quite different depending on fungus species and developmental stage of the insects (Samson, 1988). Therefore, when a particular insect pest control programme is considered using these fungi, the particular species or strains which are most suitable have to be taken into account. Similarly, dose and time of exposure

of the host to insect pathogenic fungi and the time taken to kill the host are also important parameters for evaluating the virulence of the fungi. Fungi which need shorter exposure period and kill the host quickly are very important for the success of application. Therefore, it is, important that the activity of selected fungus isolates should be screened against the particular target host at the initial stage. Such pathogenic relationships may give ideas related to virulence of the species and the fungus density necessary to kill at least 50% or more of the pest species. This chapter focuses on a general discussion of the findings of previous chapters and implications for the sustainable management of white grubs in Nepal under the following four major topics.

### 12.2.1 Study of the severity of white grubs and occurrence of entomopathogenic fungi in Nepal

White grub problems in Nepal are increasing every year for several reasons. Among them the regular cultivation of maize crops, followed by millet and rooted vegetables, without incorporation of a legume crop has largely favored the occurrence of pests. In addition, the heavy use of un-decomposed farm yard manure (FYM) has aggravated the problem every year. Monocropping coupled with the use of traditional tools during cultivation are other factors favoring the white grub's severity in upland farming. Unlike mechanized farming such equipments impose much less disturbance to the hibernating stage of the pests. The higher magnitude of the white grub problem in upland farms in comparison to lowland areas is probably associated with the soil moisture, disturbed habitat, rainfall, pH, unfavourable host crops etc. In the majority of the cases, soil pH in upland areas is comparatively higher than in the low land areas. An increasing problem of white grubs due to similar reasons is reported by Yadava and Vijayavergia (1994) in India. Although climatic conditions may be limiting for disease host density which may directly influence the rate of disease build up (Benz, 1987). Soil moisture is an important factor affecting population dynamics of white grubs (Brown and Gange, 1990). It is because certain levels of moisture in the soil are essential for egg survival and hatching and for survival of the larvae especially in their first instars (Potter and Gordon, 1984).

A first survey was initiated in the summer of 2002 and revealed that very few (0.2%) white grubs were naturally infected with the fungus *M. anisopliae*; however, more than 50% of the soil samples taken in Parbat, Tanahun and Chitwan Districts indicated the presence of the fungus under natural conditions (Chapter 3). This result demonstrated that the GBM and selective medium are two methods appropriate for detecting the presence or absence of insect pathogenic fungi and their density in the soil respectively. Therefore, it is recommended to use these methods in future investigations, either in surveys for entomopathogenic soil fungi or in monitoring the fungal distribution in the study sites. Until recent, more than eighty different strains of *M. anisopliae* and eight strains of *B. bassiana* were recovered and identified. These foundation strains can be used for further experimentation.

The insect pathogenic fungi are available in Nepalese soils and also associated with insects. They can be isolated easily and cultured on selective medium. Mass production can be done in liquid medium followed by the inoculation of locally available cheap solid substrates such as barley grains. It is hoped that such materials can be produced indigenously and in future further modifications can be adopted to make mycoinsecticides available to the

farmers for field application. These entomopathogenic soil fungi can be produced and applied against white grubs in particular and soil insect pests in general through augmentative or inundative release with the joint efforts of research, extension and private counterparts. Further, other formulations of the fungi can be used to control insect pests living in the plant canopy. However, there is a need of investment of private enterprises for its large scale production.

### 12.2.2 Virulence of different strains of *M. anisopliae* against white grubs using different bioassay methods

The selection of virulent strains is a prerequisite for an efficient control of insects using fungal pathogens. In Chapter 4, the suitability of different bioassay methods were assessed to select the virulent strains based on time and dose mortality against the white grub species prevalent in Chitwan District (*M. affinis*). As it was demonstrated, the variability of the efficacy of different strains (in this study expressed as $LT_{50}$ values) is considerably high according to Steinhaus (1949). The bioassay experiments convincingly showed that some of the isolates originating from white grubs were found to be more virulent than isolates from soils. Similarly, the age and species of white grubs coupled with fungus inoculum based on conidiospores and blastospores (Chapter 6) influence the efficacy of the biocontrol agents. In bioassay experiments, dose and time of exposure of the host insect to the fungus and the time taken to kill the host are also important parameters for evaluating virulence of insect pathogenic fungi. The dose-mortality experiment showed the delayed mortality with lower concentration of fungus spores, which corresponds with similar findings of Daoust and Roberts (1982).

Bioassays are usually conducted under laboratory controlled conditions with optimal temperature, moisture, humidity, insect species, body size, weight and instars; however, ecological fitness of a pathogen may be different under natural environmental conditions. In most parts of the terai regions of Nepal the soil temperature during the summer season may be unfavorable for the pathogen as it exceeds 30 $^{o}$C. In general, standardization of the bioassays is difficult because of the lack of suitable host insects obtained from similar conditions which are necessary for the experimentation. In our experiments, this factor was largely reduced because of the successful artificial rearing of the white grubs.

The development of commercial products based on entomopathogenic fungi for the use in integrated pest management program requires several steps. Fungal species and isolates must first be obtained from diseased insects or from the environment, and identified. Techniques for culturing and preservation are essential steps. Pathogenicity of the recovered strains needs to be screened in tierd experiments and the most promising candidates are evaluated in bioassays and then mass produced. All these findings should be taken into consideration when selecting new fungal strains for use as BCA in the field.

### 12.2.3 Mass production and field application of *M. anisopliae* for white grub control as BCA

The experiments for mass production with *M. anisopliae* in autoclavable plastic bags as described in Chapter 7 demonstrated the feasibility but also the importance of the bag quality. Fungus quality depends on both the quality of the propagules in combination with

solid substrates and bag quality. When Nepali bags were used, the solid substrates stuck together and the growth of the fungus was limited. This is attributed to an insufficient air circulation inside the poly bags as indicated by Keller (2004). Best qualities with more than 90% grains colonized with pure *M. anisopliae* were achieved on barley kernels produced in Swiss bags. Such a quality fulfills the requirement for mass production. Additional efforts must be undertaken to develop a production system which is based only on materials available on the national market. Likewise, modifications of the production system should be explored for a country like Nepal, where alternative equipments for autoclavation may be adopted like the use of pressure cookers for small scale production of the fungus. Similarly, bottles can alternatively be assessed instead of poly bags to reduce the cost of production.

Once the fungus is mass produced it is important to induce epizootics in the host population. The success of the control of the soil pests with entomopathogenic fungi is mainly dependent on the efficacy and persistence of the pathogen in the soil environment. These parameters are largely depended on the host density, soil temperature and moisture (Studdert *et al.* 1990), soil types as well as antagonistic organisms (Fargues, 1984). Generally, fungi are limited in their ability to control insect pests. For example, not all pest species are susceptible to fungal pathogens, and even if they are susceptible, the target hosts may live in an environment that is not conducive to fungal infection and transmission. As discussed in Chapter 8, fungal pathogens are highly dependent on moisture for spore germination and infection. They are also adversely affected by high temperatures. Pathogen survival in the host's environment is necessary for the long- term persistence of disease in the host population. Pathogen population density and spatial distribution are key factors in the development of an epizootic, as they affect the likelihood of contact with viable hosts (Tanada and Fuxa, 1987). For this reason, the level of fungus density in the soil may be increased by inoculative strategies (Eilenberg *et al.* 2001).

Soil moisture and host density are important factors that affect both the fungus population, (Brown and Gange, 1990) and the spread of the disease in the soil environment. Additionally, water is essential in the soil for the successful development of the eggs. Both the eggs and the larvae cannot survive below a given soil moisture thresholds (Potter and Gordon, 1984). The development of an epizootic in the host population proceeds as long as the fungus can multiply on the white grubs, but the density of the fungus decreases after a large proportion of host insects have been killed. However, in our experiment no such reduction of the fungus density was evident even after reduction of the grub density. This result may be largely due to the presence of more than one grub species in the same piece of land. The soil moisture in the earlier period of maize sowing was low; however, it progressively increased until the monsoon seasons. Looking into these two aspects, summer application of the fungus seems one of the best choices, however, soil temperature on the other hand turned out to be a major influencing factor especially in this season. In response to the later cases, we can augment the fungus either by splitting the dosages or the application of the fungus should be conducted in spring or early summer. Soil temperature during this season allows an optimal development of the inoculum in the soil. So far as the humidity is concerned for the successful development of the fungal inoculum in the soil, it would not be a major problem in Nepal if the fungus kernels are placed at 8-10 cm depth during the main season of maize sowing.

Alternative methods of application of the fungus kernels in combination with FYM may be better alternatives in Nepal since every farmer incorporates farm yard manure a few days before maize sowing. The incorporation of FYM enhances the attraction of the beetles to lay their eggs into such heaps. The attraction of beetles under such FYM is also favoured by the moisture which enhances the better site for beetle oviposition and better source of feeding materials for first hatched neonate larvae. At this period the grubs become active in the majority of the farm area. In the same way, the hand placement of fungus kernels along with the maize seed inside the bullock made furrows has primarily two advantages. It allows the localised applications which increase the chances of establishment of the fungus inoculum inside the soil. Secondly, it allows a fast contact of the fungus spores with the white grubs searching the roots of the maize seedling. Most of the farm sites in Nepal are strongly terraced and mechanised farming is not possible. In such areas the localised placement of fungus may be well achieved using bullock drawn plough and hand operated spade.

Properties of the pathogen population that are important in epizootiology include virulence and pathogenicity, dispersal and survival in the host's environment, and inoculum density and spatial distribution (Tanada and Fuxa, 1987). Atmospheric moisture is often considered the most important abiotic factor in the epizootiology of fungal diseases (Fuxa and Tanada, 1987). This factor should not be a problem in our experimental plots, where the fungus is able to acquire moisture from sources other than precipitation, such as dew and soil moisture. Further, the humidity in a dense plant canopy may be much higher than that of the ambient air (Kramer, 1980).

Sufficient number of larvae and pupae are killed by flooding (Cherry, 1984). Probably due to this reason white grubs are not as problematic a pest in lowland rice growing area as compared to rain fed area. In the low land area of Nepal, the crop is grown only after sufficient flooding and the field has been plowed many times. This type of land cultivation does not exist in upland areas. Application methods in combination with cultural practices such as summer and winter plowing and assessment are other important parameters for the success of the strategies.

In our experiment, there is neither evidence that *M. anisopliae* is able to infect and multiply on other insects than white grubs, nor is saprophytic growth outside the host range likely. Bioassays with *M. anisopliae* strains tested against non-target organisms like the earthworm (Hozzank *et al.* 2003) Collembolans (Vestergaard *et al.* 1995) revealed no fungal infections. Therefore, risks to non-target organisms after introducing massive numbers of propagules of *M. anisopliae* into the soil environment are very small. However, continuous studies on the effects on non-target organisms such as the larvae of Carabid beetles and other useful organisms in the soil environment are necessary. Long-term monitoring of the fungal population with a defined set of techniques to investigate the persistence of the released fungus strains is essential in order to improve the control method. Nevertheless, future research is needed to investigate the fate of the released strains in soils and their short- and long-term effects of the host population.

### 12.2.4 Identification of damaging species of white grubs and population dynamics in relation to microbial control

The severity of white grubs in Nepal is also intensified by the fact that there are several species of white grubs within a small geographical area. This evidence is overwhelmingly supported by the findings presented in the Chapters nine through eleven in which it is described that eighty different beetle species are recorded from four places. Additional beetle species were recorded in the fungus applications experiment, which largely suggests there are many more beetle species are present in Nepal. Different beetle species have different life cycles with consequences on crop damages. The life cycle of the white grub lasts one to three years depending on the species, available host plants and the associated environment. In some locations, crop losses occur annually, whereas in other locations they occur either biannually or every third year depending on the involved species. Identification of the damaging species and understanding of their biology are therefore the two initial steps in planning the management strategies. The experiments further revealed that the period of occurrence of the beetle is highest in the month of May and lowest in December. Therefore, management strategies should consider the flights of the beetle and the time of larval occurrence in the soil. In low land tropical areas of Nepal, the presence of large populations of annual grubs suggests that the control options may be directed to two periods of the year, one in March-April and another in June-July. The same recommendations may not be applied in mid to the higher hills of Nepal since major damages of the white grubs occur in alternate year as for example in the case of Parbat District. Looking into the species involved, different strategies for their control should be adopted; however, the microbial approach could be one of the important components of white grub control. Continuous screening of isolates from the larvae of all the locations is also important for obtaining more virulent strains. The fungus application should be promoted into endemic areas of the white grubs and application time should coincide with the pest biology. Repeated applications may be necessary to increase the fungus inoculum in the soil, which may bring down the pest population gradually and reduce the need of pesticide use.

## 12.3 CONCLUSIONS

The results of this thesis provided important information on the different aspects of the hosts, the white grubs, and the pathogen, *M. anisopliae* from which a microbial control can be initiated in Nepal. The overall conclusions of the work from exploration to exploitation of the indigenous fungus, *M. anisopliae,* for white grub control are summarized as below.

12.3.1 White grubs are nationally important insect pests in Nepal, which occur in almost all cultivated and non-cultivated area. Nearly, hundred different species are recorded from this study which overwhelmingly demonstrates the species richness. Identification and knowledge of the damaging beetle species and their biology are prerequisites for fungus application. Therefore, control options should be initiated regularly with inoculation strategy.

12.3.2 White grub damages in Nepal occur in a cyclic manner depending on the locality and species involved. In most part of terai regions similar to Chitwan and Nawalparasi Districts, white grub species such as *Maladera, Adoretus, Heteronychus, Mimela, Allisonotum* and *Anomala* are common, whereas in low mid hill area like Syangja and

Tanahun Districts, *Phyllognathus, Holotrichia* are abundant. In the same way, the grubs in high and mid hills similar to Parbat and Sindhupalchowk Districts, *Lepidiota, Xylotrupes, Sophrops* and *Holotrichia* are the main ones involved in crop damage. Because several species of beetles are involved in crop damage, location specific and species specific control options may be useful.

12.3.3 In most parts of low hill areas, various species are involved in the crop damages. Their occurrence has mainly two peaks within the year, one in March-April and the other in July-August. The grubs in mid and high hills occur in alternate years depending on the life cycle of the grubs.

12.3.4 Control operations should coincide with the life cycle of the white grub species. Two strategies such as control of adults and larvae can be adopted. In areas where electric power is available, light traps can be used as an indicator of the occurrence as well as trapping out the early population of the adults. During the breeding season, adults aggregate massively on trees, and therefore large numbers of adults may be collected by shaking them into a plastic sheet. More occurrences of larvae in following season can be expected with the massive activity of the beetle flights; therefore, control strategies can be planned accordingly.

12.3.5 Entomopathogenic fungi, *M. anisopliae* and *B. bassiana*, are present in Nepalese soils and in white grub populations, although at low density and frequency. These pathogens can easily be isolated using simple techniques, the *Galleria* bait method (GBM) and the selective medium. This finding overwhelmingly suggests that indigenous strains can be mass produced and used without delay in Nepal and import of exotic pathogens can be avoided.

12.3.6 Some Nepalese fungal pathogens are virulent to white grub since they induced mortalities of over 80 % under standard bioassay conditions in the laboratory. Furthermore, they were as virulent as Swiss isolates; therefore, they can be further promoted for mycopesticide production. The pathogens are virulent to the white grub species that are found in the damaging sites of Nepal. Therefore, they may be used irrespectively of the white grub species common in different farming systems.

12.3.7 Mass production can be initiated using local materials and equipments such as barley, low quality rice and locally available pressure cookers. The quality of the BCA can be assessed using simple techniques such as sporulation tests in simple trays with filter paper, peat or soils.

12.3.8 Unlike in mechanized farming, BCA application in Nepal can be integrated very effectively into farmers' practices during the maize sowing time. It is because the maize seeds are mainly sown in the furrows made by a bullock drawn plow which ensures localized placement of fungus kernels and maize seeds together. The fungus and maize seeds placed together will become well covered with the next furrows. In order to achieve the better results, these agents should be used in the endemic localities by inoculative release.

12.3.9 The first field applications adopting farmer's practice indicated some reduction of white grub populations which further indicates the way forward, with ample opportunity of proceeding with the fungal based mycopesticides in Nepal, targeting white grubs in particular and soil-dwelling insect pests in general. The selection of virulent strains needs standardized bioassays for specific target pest species. The difference in virulence of the strains between laboratory and field conditions should be narrowed down so far as possible.

12.3.10 In order to achieve good results, the application of the fungus-colonised kernels should be done in spring or early summer, as the development of *M. anisopliae* depends on a suitable temperature range. Prolonged control of the pests largely depends on several other factors; however, the host density has a marked effect on the establishment and survival of the fungus after application. Therefore, further research on the aspects of production and formulation of biopesticides, on recommendations of dosages is necessary for an optimal development of the fungal inoculum that might lead to a reliable control.

12.3.11 In Nepal, microbial control is an entirely new approach. It is almost at the embryonic stage. Fungi have demonstrated their capacity to naturally regulate pest populations. Our ability to capitalize this potential and develop products and strategies that exploit their unique characteristics presents a significant challenge. We have to gradually reduce the use of pesticide by the farmers with appropriate collective efforts.

## 12.4 FUTURE RECOMMENDATIONS

Although the list of research needed on fungal pathogens and their use in biological control is long, there are specific areas which need additional attentions.

12.4.1 It is important that strains of fungal pathogen are collected regularly from the wider agro-ecological zones and preserved for future evaluation. Long term preservation of the fungus strains within the country is utmost important.

12.4.2 Continued expansion of research on fungal epizootiology and host ecology is needed so that disease dynamics, the impact of pathogens on host populations, and the factors that limit disease development and spread under field conditions can be understood.

12.4.3 The genetic aspects of host and pathogen populations that affect the establishment, spread, and maintenance of disease in insect populations need to be studied. It must be recognized that natural populations of fungal pathogens contain high levels of genetic variation that could contribute to their ability to regulate host populations. Furthermore, the potential for the development of resistance in the host population must be explored before it becomes a problem in the field.

12.4.4 Efforts to integrate the use of pathogens with other control tactics must be increased. In this study, control efforts were mainly concentrated on the use of fungi. However, multiple tactics such as trapping systems and cultural techniques would be helpful in reducing the white grub problems.

12.4.5 Research should be continued to obtain more virulent strains of the fungus. Mass production can be started with virulent strains; some of them may be used from the present

collections. Mass production of insect pathogenic fungi should focus on different formulations such as powder form, granular form or liquid form in order to target the pest insects that feed in underground portions as well as on aerial plant parts.

12.4.6 The study of the pest biology and identification of damaging species across the agricultural domains should be updated in the reference collections at the Institute of Agriculture and Animal Sciences (IAAS), Rampur. Similarly, core collections of entomopathogenic fungi and future screening should be done at IAAS, Rampur.

12.4.7 As the insect pathogenic fungi are useful against a wide range of pest insects, their continuous assessment should be carried out against several soil dwelling pests such as red ants (*Dorylus orientalis)* and cut worms (*Agrotis* spp.). In the same way, they should be assessed against fruit boring pests such as *Helicoverpa armigera*, aphid, caterpillars of Cole crops such as cabbage butterfly (*Pieris brassica*) and others.

12.4.8 The use of innovative techniques in addressing white grub problems associated with biological control agents needs support from many disciplines. It is evident that specialists from many sub-disciplines of entomology, genetics, mycology, systems science, and other fields of study will be required to identify, manipulate, and use fungi successfully for biological control purposes in the future. Nepal Agricultural Research Council (NARC), IAAS, Rampur, Royal Nepal Academy of Science and Technology (RONAST) and private organisations involved in research as well as organisations involved in extension and development such as the Department of Agriculture (DOA) and non-Governmental Organisations (NGOs) may jointly carry out this kind of activity in Nepal. It is also necessary that production of these fungal agents as mycopesticides may be undertaken by private organisations in cooperation with IAAS and other research partners.

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## Biographical summary

I, Yubak Dhoj G. C., was born on 30th June 1963 as a last son of Mr Dammar Bahadur G. C. and mother Bel Kumari G. C. in Gulmi District, Lumbini Zone, Western region of Nepal. I am Hindu by birth and Chhetry by caste, but believe in work with truth and religiously I am neutral. My primary and lower secondary education were completed from Malika lower secondary school, Simaltari, Gulmi, Nepal. After my higher secondary education from Himalaya school, Dhurkot, Gulmi, I studied I. Sc. and B. Sc. Agriculture at the Institute of Agriculture and Animal Sciences (IAAS) of Tribhuvan University (TU) from 1986 to 1991 respectively at Paklihawa and Rampur campuses, Nepal.

After completing my bachelor degree in agriculture, I joined Government employment as a Livestock Officer in one of the remote hilly Districts of Nepal under the Integrated Rural Development Project (IRDP). After 3 years of service, I moved into another discipline of work in the field of horticultural under the Nepal Agricultural Research Council (NARC) based in the Agricultural Research Station (ARS) Pokhara, Nepal, where I served for about a year. While continuing this job, I then moved to Lumle Agricultural Research Centre as an Entomologist. This station was one of the Hill Agricultural Research Centers in Nepal which was reputedly known as Kande Bikas. The centre was managed by the British Government through the Overseas Development Agency (ODA) for more than three decades in Nepal. At this station, I served for more than 5 years and during this period I spent for more than 3 years in farmer's participatory research in the Western region of Nepal. This was a wonderful opportunity for me to put the theory into practice and was an immense source of motivation in setting my future career in the field of plant protection. After three years of field experiences, I was then transferred to the Lumle centre undertaking the responsibility of Integrated Pest Management (IPM) in the IPM Research team. During this period, I was awarded for Master degree study at the University of Reading, England, U. K., for which I got scholarship through the British Council from the then Overseas Development Agency (ODA) currently known as Department for International Development Fund (DFID), and returned to Nepal after completing the study at the end of September 1998.

I wanted to resume my research career in Lumle, however, the centre was already phasing out on my return. I was very keen to continue my career in research and teaching, and therefore joined IAAS, Rampur in October 1998 in the capacity of Lecturer and have been continuously associated to date in the Department of Entomology. During this University career, I continuously strived for the higher study and got an opportunity for PhD study within the framework of a research project on "Biological control of white grubs with indigenous insect pathogenic fungi in Nepal". Initially, I had started an exploratory study in the similar field since the middle of 2001; however, it was only possible to institutionalize the research into a PhD study since March 2003. I undertook the PhD program in the field of microbial control of white grubs at the University of Basel, Switzerland, with a research grant provided by Helvetas-Switzerland. This project was coordinated by the Sustainable Soil Management Programme (SSM-P) Nepal. After completing my PhD studies, I will return to Nepal and continue my teaching and research at TU/IAAS, Rampur, with fresh knowledge and great enthusiasm. I wish to serve my motherland through effective delivery of the knowledge in the University and by developing eco-friendly pest control which might be useful to food security and environmental protection in the long run.